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CONTENTS

The Role of Dual Reading in Mass Radiography. J. YERUSHALMY, J. T. HARKNESS, J. H. COPE, AND B. R. KENNEDY 443
Friedländer's Pneumonia. ROBERT H. WYLIE AND PAUL A. KIRSCHNER 465
Acute Lung Abscess. WILSON WEISEL, FORRESTER RAINE, AND GEORGE C. OWEN 474
The Resistance of Tubercle Bacilli to Chemotherapeutic Agents. DIRAN YEGIAN AND ROBERT J. VANDERLINDE 483
Experimental Studies on the Pathogenesis and Prognosis of Renal Tuberculosis. ANDREW SPORER AND MONROE E. GREENBERGER 508
The Streptomycin Treatment of Genitourinary Tuberculosis. JOHN K. LATTIMER, J. BURNS AMBERSON, AND STANLEY BRAHAM 518
The Effect of Streptomycin on the Morphology of the Tuberculous Lesion. M. CLARKE SILVERTHORNE AND GERTRUDE SILVERMAN 525
Pathology of Pulmonary Tuberculosis: As Modified by Streptomycin Therapy. HUGH W. MAHON 543
Effect of Intravenous Tuberculin Injection on Subsequent Tuberculin Skin Reactions in Hypersensitive Rabbits. CURTIS SANDAGE AND JORGEN M. BIRKELAND 556
The Distribution of Iron in Tuberculous Granulation Tissue. GEORGE GOMORI. 560
Methods for Isolating Tubercle Bacilli. E. GWYN ROBERTS, JACK L. WALLACE, AND HENRY ERLICH 563
A Direct Method for the Determination of the Sensitivity of Tubercle Bacilli to Streptomycin. GUY P. YOUMANS, ABDULLA IBRAHIM, JOAN SWEANY, AND HENRY C. SWEANY 569
Plate Method for the Determination of Streptomycin Sensitivities of *Mycobacterium Tuberculosis*. BERKELEY H. JOHNSON 578
A Comparison between an Aneroid Spirometer and a Water Spirometer. DAVID H. COHEN 582
Editorial: Tuberculosis on the Navaho Reservation. LEWIS J. MOORMAN 586
Books 592
Abstracts 69

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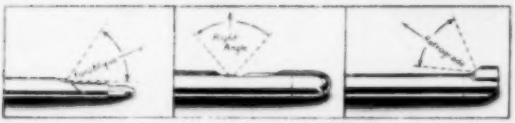

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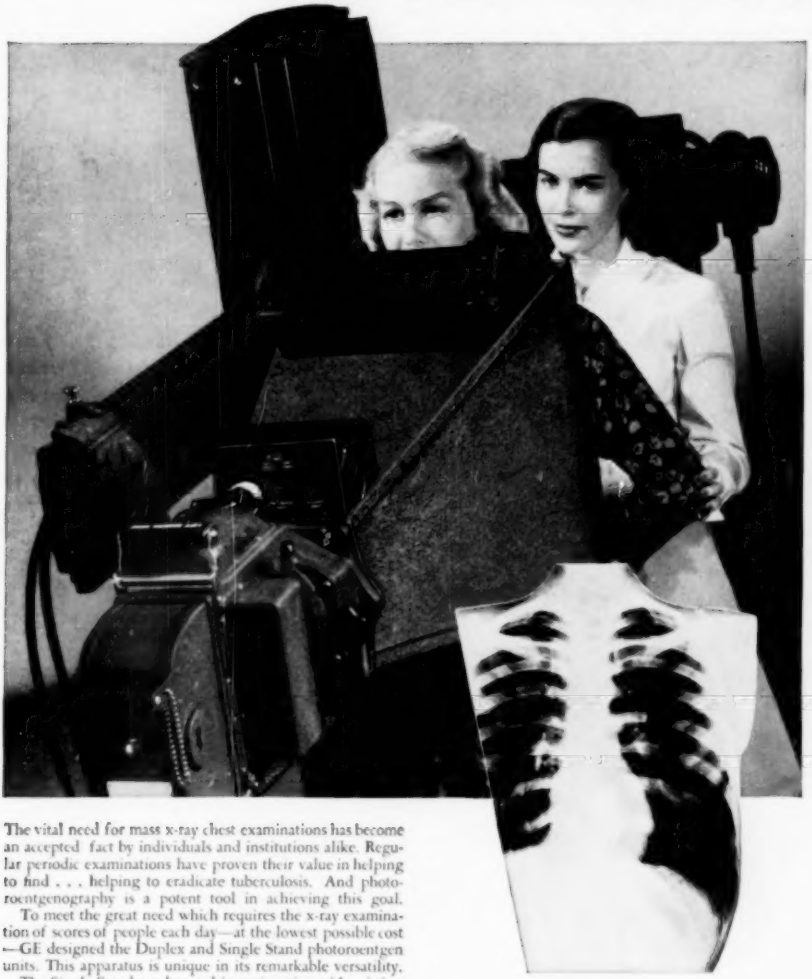

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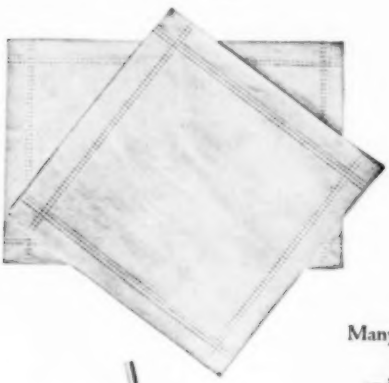

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THE ROLE OF DUAL READING IN MASS RADIOGRAPHY[1, 2]

J. YERUSHALMY, J. T. HARKNESS, J. H. COPE, AND B. R. KENNEDY

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INTRODUCTION

Many workers engaged in the conduct of mass radiography surveys have been troubled in recent years by the demonstration of subjective errors in film interpretation. It has been shown that competent radiologists and chest specialists miss as many as 20 to 30 per cent of films with demonstrable evidence of inflammatory disease (1, 2, 3, 4). Consequently, many persons throughout the country are being informed, on the basis of the interpretation of their photofluorograms, that they are free from disease when, in point of fact, they are not. Equally disturbing is the reverse situation where persons free from disease are being subjected to inconvenience, expense, and psychological trauma because their films were falsely diagnosed as showing evidence of pulmonary disease. The high frequency with which these false diagnoses occur presents a problem of great importance in the field of tuberculosis case finding.

Fundamental studies on the problem of diagnosis may in the future produce methods of film interpretation less burdened with error, but it seems wise not to wait until the complete solution is obtained. Instead, it is advisable to develop methods which make use of present knowledge to lessen the undesired effects of false diagnosis. One method for accomplishing this goal is that of dual reading of all survey films.

Dual reading means that a series of films is read and the identification numbers recorded of every film showing evidence of inflammatory disease; later the entire series is read again in the same way by the same or another reader *without knowledge* of the first readings.

One effect of dual reading is a reduction in the number of false negatives,

[1] From the Division of Biostatistics School of Public Health, and the E. V. Cowell Memorial Hospital, University of California, at Berkeley.

[2] Assistance for the study was provided in the initial phase by the Division of Tuberculosis Control, Public Health Service, and later by the San Francisco Tuberculosis Association.

that is, positive films which are diagnosed as negative. This reduction may be expected only if the two interpretations are completely independent, for an abnormal film is missed by a reader as a result of an *accidental* oversight either in perceiving an abnormality or in not attaching to the shadow the weight that it deserves. It may be assumed, therefore, that the probability that the same accidental oversight will occur on two independent readings is greatly reduced.

Unfortunately, the procedure of dual reading is a two-edged sword. While it reduces the number of false negatives, it has the opposite effect on the number of "false positives" (negative films which are diagnosed as positive). In fact, dual reading may be expected to nearly double the number of the latter because each reading will produce its own accidental "false positives."

In evaluating the usefulness of dual reading in mass radiography, it is, therefore, not sufficient to determine the number of additional positive films which will be detected by the second reading. It is equally important to determine the additional number of negative films which will be diagnosed falsely as positive as a result of the procedure. Further, it is advisable to seek ways of escaping the penalty of the latter while retaining the benefit of the former.

The present experimental study has been designed to evaluate the advantages and disadvantages of dual reading, as well as certain modifications of the procedure. It provides quantitative data on alternate methods of dual reading which may form a basis for achieving the desired balance between over-reading and under-reading.

MATERIAL AND METHOD

The material on which this study is based was provided by a set of some 1,800 photofluorograms (70-millimeter) taken routinely on all students who enrolled for the first time in the 1949 spring semester in the University of California at Berkeley. No selection was exercised other than the age, sex, and economic status inherent in a student group, since the chest photofluogram was part of the physical examination required of all entering students.

It is advisable, first, to discuss briefly two fundamental concepts involved in studies on roentgenographic interpretations of survey films.

The first is a clarification of the terms "positive" and "negative." There can be no single definition of the term "positive" which would serve all purposes. In some activities only "bacteriologically proved cases of tuberculosis" may be considered "positive." In others, clinical symptoms are used as criteria, while for certain other purposes a calcified lesion may be all that is required as a criterion for "positive." Before using the term it is therefore necessary to determine the purpose for which it is used and adopt the appropriate definition.

For the present study, the requirement is a *roentgenographic criterion* of "positive." The purpose of mass radiography is to select from among the persons examined those who have roentgenographic evidence of disease, in order that they may be referred for further clinical and laboratory studies. Consequently, it is neither necessary nor desirable to consider clinical and bacteriological evidence of disease. These are the essential elements of follow-up, not of the initial

stage of case finding. In the present study, therefore, the term "positive" refers to *a person who has roentgenographic evidence of inflammatory disease significant enough to require further study.*

The second complication is inherent in the fact that for the present study it is necessary to determine which of the 1,800 students are "positive," that is, possess roentgenographic evidence of disease. Without this knowledge it is not possible to evaluate the performance of a single reader or group of readers. The difficulty arises from the fact that *a priori* this information is not available. The knowledge as to which person in the group is "positive" and which one is "negative" must come from an interpretation of the films, the very activity of which is under investigation.

The method employed in the present study for determining the "positives" as reliably as possible was, first, to attempt to identify every film which might possibly possess an abnormal shadow. This was accomplished by *subjecting the films to ten independent interpretations.* The probability that a "truly positive" film will fail to be detected in any of these interpretations is obviously very small.

This procedure produces a very large number of suspected cases, the vast majority of which are negative. It now becomes necessary to identify the "positives" in this large group of suspects. This was accomplished by obtaining a 14 by 17 celluloid film on each of the suspects and having these interpreted by a group of experts independently and in conference. The films selected by *all* of the readers as possessing evidence of inflammatory disease requiring clinical follow-up were considered "positive" for the purposes of the present study.

In all, there were 1,816 photofluorograms. These were interpreted by six qualified radiologists and chest specialists designated A, B, C, D, E, and F. The first four provided two readings each, the last two only one reading. Thus, a total of ten independent readings of the set of films is available.

Two of the readers (A and D) previously had had little or no experience in reading survey photofluorograms. It was found, however, that their interpretations did not differ materially from those of the remaining four who had had long and continuous experience, and therefore all interpretations were included in the analysis.

There were eight films which were subsequently found to represent nontuberculous disease and therefore were excluded from the present study. Another case was eliminated because a transient "pneumonic" process which was present at the time of the 70-millimeter film had cleared by the time the 14 by 17 film was taken. The present study is therefore based on 1,807 films.

As a result of the ten independent interpretations, 254 photofluorograms were called "positive" on one or more of the readings. The 254 students were recalled for 14 by 17 celluloid films. These were interpreted independently six times. If all six readings were negative, the case was so considered. The rest were interpreted independently and in conference by seven radiologists and chest specialists. Films which were interpreted by *unanimous opinion* of all the seven readers as showing evidence of inflammatory disease requiring clinical follow-up

were considered positive. There were 30 such cases and these will be referred to in the present study as "truly positive." In addition, there were twelve films in which the majority opinion was for follow-up, and five with majority opinion against follow-up. These are considered "questionable." In the remainder, opinion was unanimous against follow-up. These will be referred to as "truly negative."

In this way 30 cases were classed "truly positive" and 1,760 "truly negative." The 70-millimeter films of those 1,790 persons form the material of this study. The arbitrary exclusion of 26 of the original 1,816 photofluorograms (9 known nontuberculous and 17 with divided conference opinion) makes the analysis much more clear-cut, for now a given reading can always be classed as right or wrong.

It is not to be supposed that every one of the 1,760 "truly negative" is indubitably free of disease, nor that each of the 30 "truly positive" is suffering from clinically active tuberculosis. Nevertheless, since each of the 30 has unanimous agreement on the part of seven experts that he needs clinical study and follow-up, it cannot be doubted that the purpose of the photofluorographic survey was to discover exactly these 30 cases.

GENERAL FINDINGS

Variations in individual interpretations: Before proceeding with the evaluation of dual readings, it may be advisable to indicate the extent of individual variation found among readers when they interpret a set of films.

Table 1 shows that the number of films called positive (out of 1,807 photofluorograms, not yet reduced by deletion of those ultimately called "questionable") varied with the reader from 32 to 83, and even between readings by the same reader to a lesser, though still considerable, degree. Moreover, the ones called positive by one reader were often not the same cases which were called positive by another reader, so that, in all, 254 films were called positive at least once. This still does not tell how good a job each of the readers has done, whether the survey was better served by the one who read 32 positives or the one who read 83. To discover this, the records of each reader must be checked against the "truly positives," "truly negatives," and "questionables" of the group conference. As stated above, the 17 questionable cases were at this point deleted entirely.

The performance of each reader in his first reading on the 30 "truly positive" films and on the 1,760 "truly negative" films is shown in table 2. It is seen that none of the readers selected all of the 30 "truly positive" cases. The most successful readers selected 22 of the 30 cases; the least successful, only 17. In other words, a single reader at best missed 8 of the 30 "truly positives" in the group. The number of misses was as high as 13 out of 30. On the average, a reader of the group missed 9.7 or 32.2 per cent of the "truly positive" films.

Table 2 also shows the number of positive diagnoses which each reader gave on the 1,760 "truly negative" films. The smallest number of false positives was 15 and the highest 51. On the average, the readers over-read 29.7 films

apiece, or 1.7 per cent of the 1,760 "truly negative" films. In relative terms, the problem of over-reading (false positives) is of a much lower magnitude than that of under-reading (false negatives). The highest percentage of over-reading was

TABLE 1

Individual Variation in Film Interpretation

Number of films called positive by each reader on the 1,807 photofluorograms (70-millimeter) on his first and second reading.

READER	NUMBER CALLED POSITIVE ON	
	First reading	Second reading
A	58	74
B	38	32
C	48	56
D	83	57
E	51	—
F	80	—
Range	38 to 83	32 to 74
Total number positive on one or more readings.....		254

TABLE 2

Under-reading and Over-reading

Number and per cent of false negative diagnoses on the 30 "truly positive"* films (under-reading) and number of false positive diagnoses on the 1,760 "truly negative"* films (over-reading) given by each reader on his first reading of the 70-millimeter photofluorograms (exclusive of 17 questionable films).

READER	OF ALL 1,790 FILMS NUMBER CALLED		OF THE 30 "TRULY POSITIVES"* NUMBER CALLED		OF THE 1,760 "TRULY NEGATIVES"* NUMBER CALLED		PER CENT	
	Positive a	Negative b	Positive c	Negative d	Positive e	Negative f	Under-reading of "truly positives"* (d/30) × 100 g	Over-reading of "truly negatives"* (e/1,760) × 100 h
A	51	1,739	22	8	29	1,731	26.7	1.6
B	32	1,758	17	13	15	1,745	43.3	0.9
C	37	1,753	21	9	16	1,744	30.0	0.9
D	69	1,721	18	12	51	1,709	40.0	2.9
E	43	1,747	22	8	21	1,739	26.7	1.2
F	68	1,722	22	8	46	1,714	26.7	2.6
Mean.....	50	1,740	20.3	9.7	29.7	1,730.2	32.2	1.7

* For definition of "truly positive" and "truly negative" see text.

2.9 of the 1,760 negative films while the lowest percentage of under-reading was 26.7 of the 30 "truly positive" films.

Variations in dual readings: When a set of films is read independently twice,

either by the same reader or by two different readers, the films are divided into essentially three groups: (*1*) those which were read negative on both occasions, (*2*) those which were read positive on both occasions, and (*3*) those in which the two readings were contradictory, that is, one was positive and one was negative. If there were no variations in film interpretation, all the films would fall into the groups with either two negative or two positive readings. The greater the variations, the larger will be the group with one positive and one negative reading.

First, it may be well to determine the extent of variation in the two readings themselves, that is, without reference to the knowledge available about the 30 "truly positive" and the 1,760 "truly negative" films. Any two readings on the 1,807 films provide 1,807 comparisons. These can be either *consistent* (the readings are either both positive or both negative), or *inconsistent*, one being positive and one negative. The inconsistent readings may be related to the total number of comparisons to yield an over-all *measure of inconsistency*. Such a measure will generally be of low magnitude, due to the very large number of apparently normal films on which both readings were negative.

The inconsistent readings may also be related, not to all of the 1,807 comparisons, but only to those films for which there was at least one positive reading. In so doing, a measure will be obtained which greatly overstates the degree of inconsistency since the readers are not given credit for the large number of normal cases in which they were in agreement. Nevertheless, such a measure may occasionally be desirable and actually preferred to the other because the purpose of mass surveys is to select the very few positives from among the large number of negatives. Consequently, if a significant proportion of these positives provides contradictory readings, the problem is of much greater magnitude than that which would be implied by a measure of inconsistency based on all films.

In table 3, the interpretations on the 70-millimeter films of each pair of readers is compared as to inconsistency on both criteria discussed above. The meaning of the table may be illustrated by explaining the figures on the first row, which compare the interpretations of readers A and B. When these two readers interpreted the 1,807 films, they agreed that the film was essentially normal in 1,732 cases. They agreed in 19 cases that the film showed evidence of disease and they disagreed in 56 cases, that is, one of them called the film positive and the other called it negative. The two of them, therefore, have selected a total of 75 films on which one or the other, or both of them, saw some abnormality. In 19 of the 75, they agreed and in 56 cases they disagreed.

If the 56 disagreements are related to the 1,807 total comparisons, the percentage disagreement is only 3.1. If, on the other hand, these same 56 disagreements are based on the 75 which were called positive by at least one reader, then they were inconsistent with each other to the extent of 74.7 per cent.

A review of the two last columns shows that the percentages of inconsistency between two readers, when based on the total 1,807 comparisons, vary from a low of 2.5 to a high of 5.4. When the comparison is based on the films which

were called positive at least once, the percentages of disagreements are very high indeed. The lowest was 65.8 and the highest 79.6 per cent. On the average, the per cent disagreement for a pair of readers was 3.8 when based on all films, and 74.3 when based on only such films as were called positive at least once. In other words, in only one-fourth of the cases which were called positive by at least one reader were the two readers in agreement.

The four readers who read the films twice each provide data for comparing

TABLE 3

Inter-individual Variation

Comparison of the first readings on the 1,807 photofluorograms (70-millimeter) of each reader with those of every other reader and the per cent disagreements between readers based on all films and on films which were called positive by at least one of the two readers.

READERS	NUMBER OF FILMS ON WHICH THE TWO READINGS WERE			NUMBER CALLED POSITIVE BY AT LEAST ONE READER (b + c)	PER CENT DISAGREEMENT BASED ON	
	Both negative	One positive, one negative	Both positive		All films $\left(\frac{b}{1807}\right) \times 100$	Films called positive by at least one of the two readers $\left(\frac{b}{d}\right) \times 100$
	a	b	c	d	e	f
A and B	1,732	56	19	75	3.1	74.7
A and C	1,728	54	25	79	3.0	68.4
A and D	1,694	87	26	113	4.8	77.0
A and E	1,724	59	24	83	3.3	71.1
A and F	1,694	90	23	113	5.0	79.6
B and C	1,742	46	19	65	2.5	70.8
B and D	1,709	77	21	98	4.3	78.6
B and E	1,738	51	18	69	2.8	73.9
B and F	1,712	74	21	95	4.1	77.9
C and D	1,706	73	28	101	4.0	72.3
C and E	1,735	47	25	72	2.6	65.3
C and F	1,709	70	28	98	3.9	71.4
D and E	1,701	80	26	106	4.4	75.5
D and F	1,678	97	32	129	5.4	75.2
E and F	1,703	79	25	104	4.4	76.0
Mean	1,713.7	69.3	24	93.3	3.8	74.3

the two independent interpretations of the same reader. A study of these comparisons (not presented here) shows that, on the average, when a single reader interprets a set of films independently twice, he is likely to disagree with himself in 2.7 per cent of all the films and in 61.8 per cent of the films which he considered positive at least once.

On the whole, the degree of inconsistency is somewhat greater when a comparison is made between readers (inter-individual variation) than when the comparison is for one individual with himself (intra-individual variation). In either case, however, the inconsistency is of very large magnitude, especially when

based on films which are called positive at least once. Again it should be noted that it is the latter comparisons which are the most disturbing, since the purpose of the survey is to select the relatively few films with a positive diagnosis.

Evaluation of Dual Reading

The percentages of disagreement between two readings presented above may be taken as crude measures of the subjective errors in film interpretation, but they do not indicate the benefits of dual readings. In order to assess the probable accomplishments of dual readings in terms of under- and over-reading, it is necessary to relate them to the 30 "truly positive" and the 1,760 "truly negative" films. The benefits of dual reading can then be determined in terms of the additional "truly positive" films which will be selected by two readers as compared to a single reader. The penalty paid will be indicated by the number of additional false positives which will be so selected. The results of such comparisons are presented below.

Benefits of dual reading: In table 4 are presented for each of the six readers the number of films which he himself detected from among the 30 "truly positive." In addition, it is shown how many more of these "positive" films which the original reader failed to detect were found by the addition of the readings for each of the other five readers.

As an illustration, reader A, in his initial readings of the 70-millimeter films, identified as positive 22 of the 30 films which are considered in this discussion to be "truly positive." (These, of course, were not all of the films which reader A called positive on his initial reading, for he also designated as positive a certain number which are in the "truly negative" and "questionable" groups). When the readings of B were added to those of A, one additional case from among the "true positives" was identified. If the second reading had been by reader C, however, 3 cases would have been added; if by D, 1 case; if by E, 2 cases; and if by F, 4 cases. On the average 2.2 "truly positive" films are added by a second reading to the 22 which were selected by reader A.

The last row in the table gives the average figures for the group and may be utilized to estimate what may be expected from dual readings in terms of detecting additional positive films. Thus, on the average, a single reader of the group detected 20.3 from among the 30 "truly positive" films. The addition of a second reader contributed 3.3 additional positive films from among those which were missed by the first reader. In other words, a second reading will produce about one-sixth as many more of the "truly positive" films as will be detected by a single reading. Another way of looking at it is that a second reading detects about 34 per cent of the "truly positive" films which are missed by a single reading (3.3 out of 9.7).

A number of additional points of interest are brought out in table 4. In the first place, it will be seen that even two readers do not detect all the 30 "truly positive" films. On the average, two readers select only 23.6 of the 30 and miss more than 6, or one-fifth, of the "truly positive" films. The largest number of

positive films selected by any two readers was 26 which were found in the joint readings of A and F and readers E and F.

A second point of interest is that the different readers do not contribute the same number of additional "truly positive" films. One reader added only 1.2 additional positives while two others added as many as 4.6. Readers who have selected the largest number of "truly positives" when they read singly have also added the most to the other readers when they were members of a team in dual readings, and conversely.

Another interesting point is that a similar analysis (not shown here) for the two readings of the same reader gives results essentially similar to the above. In other words, *there appears to be little difference whether the second reading is performed by the same or by a different reader, provided the two readings were performed independently.*

TABLE 4

Benefit of Dual Reading of 70-millimeter Photofluorograms

Number of "truly positives"* detected by a single reader† and number of those missed that were found by a second reader† out of 30 "truly positives"* among 1,760 "truly negatives"* (exclusive of 17 questionable films).

READER	"TRULY POSITIVES"* DETECTED							MEAN NUMBER OF "TRULY POSITIVES"* ADDED BY DUAL READING
	By single reader	Additional, if dual reading was by reader						
		A	B	C	D	E	F	
A	22	—	1	3	1	2	4	2.2
B	17	6	—	5	2	7	6	5.2
C	21	4	1	—	1	4	4	2.8
D	18	5	1	4	—	6	5	4.2
E	22	2	2	3	2	—	4	2.6
F	22	4	1	3	1	4	—	2.6
Mean	20.3	4.2	1.2	3.6	1.4	4.6	4.6	3.3

* For definition of "truly positive" and "truly negative" see text.

† Based on the first readings only.

Disadvantages of dual readings: It now becomes important to consider what penalty is paid by dual reading for the additional positive films that it detects. This is measured in terms of the additional false positives diagnosed by the second reading, because it would cause unnecessary anxiety to persons who do not possess roentgenographic evidence of disease. This information is available in table 5. The meaning of this table will become clearer by following any row, as, for example, that headed "Reader A."

It may be seen that reader A, on his first reading, designated 29 false positives; that is, he gave positive readings on 29 films which are known to be "truly negative." When the readings of reader B were added to those of reader

A there were 15 additional false positives. Reader C added 14 false positives; reader D, 47; E, 20; and F, 45.

On the average, a single reader selected 29.7 false positives and the second reader added an average of another 27.8. The number of false positives is therefore almost doubled by the addition of another reader.

From the data in tables 4 and 5, it is seen that a single reading produced 20.3 "truly positives" and 29.7 false positives. Dual reading produced 23.6 "truly positives" and 57.5 false positives. In table 5 it is shown also that *for every additional "true positive" selected by dual reading 8.5 "false positives" will be added.*

TABLE 5

Increase in False Positives arising from Dual Readings of 70-millimeter Photofluorograms

Number of false positive diagnoses given on the 1,760 "truly negative"* films by each reader† and the number of films with false positive diagnoses added by the readings of each of the other readers (exclusive of 17 questionable films).

READER	NUMBER OF FALSE POSITIVE DIAGNOSES							MEAN NUMBER OF FALSE POSITIVES ADDED BY OTHER READERS
	By single reader	Additional, if dual reading was by reader						
		A	B	C	D	E	F	
A	29	—	15	14	47	20	45	28.2
B	15	29	—	16	49	21	44	31.8
C	16	27	15	—	49	20	42	30.6
D	51	25	13	14	—	18	41	22.2
E	21	28	15	15	48	—	45	30.2
F	46	28	13	12	46	20	—	23.8
Mean	29.7	27.4	14.2	14.2	47.8	19.8	43.4	27.8
Mean number of "truly positives"* added by dual reading (table 4)								3.3
Ratio of added false positives to added "true positives"								8.5:1

* For definition of "truly positive" and "truly negative" see text.

† Based on the first readings only.

Methods of Utilizing Dual Readings

The information presented above may be used to determine the benefits of dual reading, and the penalty paid for it when such readings are used, by referring for further study every case which was called positive *on one or the other* of the two readings. This, however, is only one way in which dual reading may be used. It is the method which will succeed in detecting the largest number of "truly positives" possible by dual reading, but it is also the one which would produce the largest number of false positives.

Certain modifications may be considered. For example, instead of referring for further study all cases called positive by one *or* the other of the two readers, only cases which were called positive on both readings may be referred for

further study. Other methods relate to a panel review of the 70-millimeter films with contradictory diagnoses, with the hope that it will succeed in rejecting a large number of the false positives while retaining most of the "truly positives." Such a panel may consist of several readers, or it may be sufficient to have the 70-millimeter films with contradictory diagnoses reviewed by a single reader. The present study provides data to test these methods, indicating the favorable and unfavorable results which may be expected of each.

Films called positive on both readings: In table 6 is presented the yield in "truly positives" and in false positives of films that were read as positive on *both* of two readings.

TABLE 6

"Truly Positives" and False Positives Diagnosed by Both Readers*

Number of films (70-millimeter) out of the 30 "truly positives"* detected and number of films out of the 1,760 "truly negatives"* on which false positive diagnoses were given by a single reader† and by each pair of readers (exclusive of 17 questionable films).

READERS	SINGLE READING		DUAL READING (CASES WHERE THE TWO READERS AGREED)											
	Number of "truly positives"* detected	Number of "truly negatives"* diagnosed as positive (false positive)	Number of "truly positives"* detected also by						Number of "truly negatives"* diagnosed as positive (false positive) also by					
			A	B	C	D	E	F	A	B	C	D	E	F
A	22	29	—	16	18	17	20	18	—	0	2	4	1	1
B	17	15	16	—	16	16	15	16	0	—	0	2	0	2
C	21	16	18	16	—	17	18	18	2	0	—	2	1	4
D	18	51	17	16	17	—	16	17	4	2	2	—	3	5
E	22	21	20	15	18	16	—	18	1	0	1	3	—	1
F	22	46	18	16	18	17	18	—	1	2	4	5	1	—
Mean.....	20.3	29.7	17.1						1.9					

* For definition of "truly positive" and "truly negative" see text.

† Based on the first readings only.

In order to understand the meaning of table 6 it may be well to follow the figures for one of the readers. For example, reader A when performing as a single reader on the 70-millimeter films detected 22 of the "truly positive" films. He also gave false positive diagnoses on 29 of the 1,760 "truly negative" films. When the readings of B were added to those of A, it was found that among those that *both* A and B called positive there were 16 of the "truly positives" and there were no "truly negative" films which were diagnosed as positive by both A and B.

The joint readings of C and A produced 18 of the 30 "truly positive" and 2 false positives. The corresponding figures for the films called positive by both D and A were 17 and 4, respectively; for E and A, 20 and 1; and for F and A, 18 and 1.

The last row in table 6 presents the average performance of the group. It may be seen that a reader performing singly detects, on the average, 20.3 of the "truly positives" and gives false positive diagnoses on 29.7 of the "truly negatives." Among the films which were called positive also by a second reader are found only 17.1 of the "truly positives," but the number of false positive diagnoses on "truly negative" films has been reduced to only 1.9.

In other words, a single reader will recall 50 people and will detect 20.3 "truly positives." Two readers will have in agreement positive readings on 19 films, 17.1 of which will prove to be "truly positive."

It may therefore be seen that films which were read as positive by two readers have a much greater chance of being "truly positive" than are those which are called positive on the 70-millimeter film by only one reader. In fact, very few of those which are called positive by two readers will be found to be negative on further study. The penalty paid for this great reduction in false positives is that the number of "truly positives" detected by this method is even smaller than that selected by a single reader. By demanding agreement by the two readers, the ratio of false positives to "truly positives" is reduced to 1 in 9, but at a cost of missing nearly one-sixth of the "truly positives" detected by a single reader.

Panel review of films with contradictory diagnosis: The methods presented above are characterized by the fact that there is a certain penalty which is paid for the benefit derived from dual reading. The first method, that of referring for study all films which were diagnosed as positive on *one or the other* of the readings, succeeds in detecting more of the "truly positive" films but has the drawback that it nearly doubles the number of negative films which are referred as positive. The second method, that of referring for study only such films as were diagnosed as positive on *both readings*, has the advantage of reducing greatly the number of false positives. The penalty paid for that, however, is that a larger number of the "truly positives" will be missed.

Attempts to retain as much as possible the benefits of dual readings without incurring the associated penalties lead to investigating methods of review-readings of the 70-millimeter films with contradictory diagnoses. The rationale behind such a procedure may be formulated somewhat as follows.

When the two independent readings of a set of survey films are available, the films distribute themselves in three categories. One consists of films in which both readings are negative, the vast majority of which are "truly negative." Included in this group are a few "truly positives" which were missed by the two readers. These cannot be salvaged by dual reading since they have not been identified by either reader. The second group consists of films which were read positive on both occasions. The vast majority of these will be "truly positive" and there can be no question that the persons concerned should be referred immediately for further study and follow-up.

The third group consists of films with contradictory diagnoses, one positive and one negative. This group will contain perhaps 10 to 15 per cent "truly positive" films. If this entire group is referred for further study, the "truly

positives" will, no doubt, be detected, but in the process a relatively large number of people will have to be examined and later discharged. The purpose of having a panel review the 70-millimeter films in this last group is the hope that it will succeed in selecting all, or nearly all, of the "truly positive" films, while eliminating a considerable number of the false positives.

The material on which the present study is based offers an opportunity to investigate whether the procedure as outlined above will produce the desired results. For this purpose, all films which had contradictory diagnoses in any two readings were reviewed by a panel of three readers. The panel was told that the films were previously diagnosed as positive by one reader and negative by another, and that the purpose of the panel was to attempt to sift out the "truly positives" from among the false positives.

The method used in the panel review was as follows: Each reader viewed the questionable 70-millimeter films and recorded his diagnoses without knowledge of the readings of the other members. After all three had completed the recording of their individual findings, the case was discussed and the opinion of the group was recorded. In the majority of instances, the opinion after discussion was unanimous. In an appreciable number of cases, however, the panel could not reach a unanimous opinion.

From the information made available by this procedure, it is possible to evaluate the performance of the panel in several ways which may be useful guides on methods of utilizing the procedure. Different courses of action become possible from a panel review of questionable survey films. Under one scheme every film on which the panel was not unanimous that the film was "negative" is referred for further study. Under another, only films on which the panel reached a unanimous opinion of "positive" are referred. A third would refer for further study all those which a *majority* of the panel considered "positive."

A review of the results of the readings indicates that only the first and second method warrant presentation in this paper. In other words, when the opinion of the panel was divided, the yield in "truly positives" was about the same whether the majority opinion was "positive" or "negative."

The available data make possible also an evaluation of the performance of a single reader acting as a reviewer of the questionable survey films.

The analysis of the review readings bearing on the different methods of utilizing a panel is presented below.

Panel of three readers: The procedure of having questionable survey films reviewed by a panel before final disposition appears to be successful in achieving the desired result. This may be seen first from the following general findings.

The panel reviewed a total of 242 films on which one pair or another of the original six readers gave contradictory diagnoses. Included in this group were 20 "truly positive" films. Upon review, only 140 of the 242 were retained by the panel as "positive." Included among the 140 were 17 of the 20 "truly positive." In other words, by the use of the panel more than half of the false positives' were rejected while the majority of "truly positive" films were retained.

These general findings cannot be immediately applied since the original con-

tradictory diagnoses were provided by ten independent readings. With the data available from the present study, however, it is possible to simulate as nearly as possible what may be expected to happen when only two readings are available. For example, in the interpretations of readers A and B, there were 51 films with contradictory diagnoses (exclusive of questionable films). Included in these

TABLE 7

Panel of Three

Number of "truly positives"* detected and false positives eliminated by a panel of three from among the films with contradictory diagnosis by each pair of two readers (exclusive of 17 questionable films).

READERS	NUMBER OF FILMS ON WHICH THE TWO READERS GAVE CONTRADICTORY DIAGNOSES (ONE POSITIVE, ONE NEGATIVE)			NUMBER OF FILMS ON WHICH PANEL DIAGNOSIS WAS								
				Positive						Negative		
				By at least one member			By unanimous opinion			By unanimous opinion		
	Total	"Truly positive"*	"Truly negative"*	Total	"Truly positive"*	"Truly negative"*	Total	"Truly positive"*	"Truly negative"*	Total	"Truly positive"*	"Truly negative"*
A and B	51	7	44	34	7	27	21	7	14	17	0	17
A and C	48	7	41	32	7	25	23	7	16	16	0	16
A and D	78	6	72	48	6	42	31	6	25	31	0	30
A and E	52	4	48	31	4	27	18	2	16	22	0	21
A and F	81	8	73	50	7	43	33	7	26	31	1	30
B and C	37	6	31	24	6	18	18	6	12	13	0	13
B and D	65	3	62	34	3	31	21	3	18	32	0	31
B and E	45	9	36	27	9	18	19	7	12	19	0	18
B and F	64	7	57	36	6	30	28	6	22	28	1	27
C and D	68	5	63	38	5	33	27	5	22	31	0	30
C and E	42	7	35	27	7	20	20	5	15	16	0	15
C and F	61	7	54	33	6	27	25	6	19	28	1	27
D and E	74	8	66	40	8	32	28	6	22	36	0	34
D and F	93	6	87	50	5	45	35	5	30	44	1	42
E and F	73	8	65	43	7	36	32	5	27	31	1	29
Total.....	932	98	834	547	93	454	379	83	296	395	5	380
Mean.....	62.1	6.5	55.6	36.5	6.2	30.3	25.2	5.5	19.7	26.3	0.3	25.3
Per cent of "truly positives"* detected.....				94.9			84.7					
Per cent of false positives eliminated.....				45.6			64.5					

* For definition of "truly positive" and "truly negative" see text.

51 films are 7 "truly positive." In the course of reviewing the entire set of 242 films, the panel obviously diagnosed these 51 films. It therefore becomes possible to determine what the panel's judgment was on these films. In other words, how many of these did they call negative and how many of the "truly positive" films did they detect. This information is presented in table 7.

As was stated above, the panel was not unanimous in its appraisal of a given

film in every case. In table 7, therefore, the performance of the panel in three different groups is presented: films on which the panel was unanimous that the persons concerned required follow-up; all films which were considered "positive" by at least one member of the panel; and films on which the panel was unanimous that they revealed no evidence of disease requiring follow-up.

Again, it would probably be simpler to explain the meaning of table 7 by following the figures for any two readers, for example, A and B. The first three columns show that A and B had 51 films with contradictory diagnoses, of which 7 were "truly positive" and 44 "truly negative." (Five questionable films are excluded.) When the panel reviewed the 51 films there was unanimous opinion for follow-up on 21 of these films. Included in the 21 were all of the 7 "truly positives." If all the films on which the panel was of divided opinion are included, the total number retained as "positive" was 34 which, of course, also included all the 7 "truly positives." The panel rejected unanimously 17 of the 51 and these happened to be all "truly negative." Judging by its performance on the contradictory readings of A and B, the panel succeeded in selecting all the "truly positives" and in greatly reducing the number of false positives.

The performance of the panel was not quite so successful on all of the other combinations of two readers, as may be seen from following the various rows in table 7. A fairly reliable estimate of what may be expected from the performance of a panel may be obtained from a review either of the row headed "total," which combines the findings on the 15 pairs of two readers, or from the last row which presents the average performance on any two readings.

From the latter it is seen that, on the average, two readers gave contradictory diagnoses on 62.1 films which included 6.5 "truly positives" and 55.6 "truly negatives."

Upon review of these films, the panel was unanimous for "follow-up" on 25.2 which included 5.5 of the 6.9 "truly positives." In other words, by referring as positive only to cases in which the panel was unanimous for "follow-up," 85 per cent of the "truly positive" cases contained in the group with contradictory diagnoses will be selected, while the number of false positives will be reduced from 55.6 to 19.7, a reduction of 65 per cent.

If films in which the panel was of divided opinion were included, then 36.5 persons would, on the average, be referred for follow-up. Included in this group would be 6.2 of the 6.5 "truly positives." In other words, 95 per cent of the "truly positives" included in the group with contradictory diagnoses would be detected, but the reduction in false positives would now be only 46 per cent (from 55.6 to 30.3).

The accomplishments which may be expected from a panel of three may now be summarized by adding to the above figures those in which the original two readers were in agreement in their positive diagnoses (table 6).

By utilizing a panel of three (unanimous opinion), the total number of "truly positives" detected was as follows: 17.1 from among those read positive by both readers, and 5.5 from among those with contradictory diagnoses, a total of 22.6 of the 30 "truly positives." The number of "false positives" would be 1.9 plus 19.7, a total of 21.6.

The corresponding figures, produced when the films on which the panel was in disagreement are included, are 23.3 "truly positives" detected, while 32.2 "truly negatives" will be falsely diagnosed as positive.

"Panel" of one: The results shown above for a panel of three indicate that it is an efficient procedure in the sense that it selects nearly all the "truly positive" cases which are contained in the films that were diagnosed as positive by one or the other of two readers, and it reduces the number of false positives to an even lower figure than would be diagnosed by a single reader. In day-by-day practices, however, it is difficult to assemble a panel of three experts to review the films together. It was, therefore, thought desirable to investigate what could be accomplished by a single reader reviewing the questionable films. Such an evaluation is possible because each member of the panel recorded his appraisal of the films prior to the general discussion by the entire panel.

There were five members who participated in these panels of three. The

TABLE 8

"Panel" of One

Per cent "truly positives"* detected and false positives eliminated by each of five reviewers acting as a "panel" of one on the films with contradictory diagnosis (exclusive of 17 questionable films).

REVIEWER	PER CENT "TRULY POSITIVES"* DETECTED	PER CENT "FALSE POSITIVES" ELIMINATED
A	79.2	72.4
C	74.0	50.9
D	100.0	49.0
F	90.0	51.1
G	41.7	87.2
Mean	78.0	58.5

* For definition of "truly positive" and "truly negative" see text.

performance of each member when acting as a "panel" of one on the contradictory films is shown in table 8.

It may be seen that, with the exception of one (G), an individual reader is fairly successful in detecting a large proportion of the "truly positive" films and in rejecting at least one-half of the false positives.

On the average, a single reviewer detected more than three-fourths of the "truly positives" and rejected nearly three-fifths of the false positives.

The procedure of referring out all cases diagnosed as positive by both readers and submitting the films with contradictory diagnoses to review by a "panel" of one reader will detect on the average 22.2 of the 30 "truly positives" and will result in false positive diagnoses on 25 films.

The performance of an individual reviewer may accomplish better results than the figures just quoted which are for the average of the five readers. As is seen in table 8, there are wide differences between readers. Some of the reviewers appear to have greater ability to perform such a task than do others. For example,

reader D succeeded in selecting 100 per cent of the "truly positives" contained among the films with contradictory diagnoses, and in reducing by one-half the number of false positives.

If the films with contradictory diagnoses are to be reviewed by a single reader, it is very important to select the reviewer with great care. Methods of testing need to be developed which will select the readers best qualified to act as reviewers.

DISCUSSION

The problem of false diagnosis of survey films is of such large magnitude that it should not be ignored or minimized by those engaged in mass radiography projects. A diagnostic procedure which misses as many as 30 per cent of the positives cannot be considered efficient. Great efforts must therefore be exerted to study the phenomenon and to seek solutions of the more basic problems involved in film interpretation. Until such solutions are found, the harmful effects may be reduced by submitting all survey films to dual reading. Such a procedure will not succeed in detecting all the positives but it will add one-sixth as many more positives, or about 34 per cent of those missed by a single reading. This is a significant improvement to justify the adoption of dual readings as a routine procedure in mass roentgenographic surveys.

When dual reading is adopted, that is, when the survey films are interpreted independently by two readers, the question arises of how these readings can be used most efficiently, for dual reading in its most elementary form has the defect of increasing the number of false positives. Certain refinements of methods of applying dual reading, which may be helpful, were therefore developed in this study.

There can be no single method of utilizing the procedure of dual reading which would be applicable to all circumstances. The same method cannot reasonably be applied in a survey of a highly exposed group, as in a survey of the general population. Other factors to be considered relate to the availability of medical and hospital facilities for the clinical evaluation of suspects and treatment of proved cases of tuberculosis. The temperament of the population and its education in regard to tuberculosis may properly be factors.

In the final analysis, the method of choice must be determined on the basis of an individual judgment as to the relative importance of false negatives compared to false positives. Three attitudes may be considered:

(*1*) The missing of a "truly positive" case is so serious that it dwarfs the importance of false positives. It is justifiable to bother any number of persons to avoid missing a positive case.

(*2*) An excessive number of false positives may jeopardize an entire case-finding program. It is justifiable to miss a somewhat larger number of "truly positives" if the number of false positives can be greatly reduced.

(*3*) A balanced judgment; the disadvantage of bothering needlessly a certain number of persons is evaluated in the light of the advantages of early detection of a positive case and the resultant saving in length of sanatorium care.

The present study, obviously, was not designed to evaluate the merits of these attitudes. It does, however, provide quantitative data on methods of utilizing dual readings which would fit into one or another of the three attitudes. In a very general way it may be stated that:

If attitude (*1*) prevails, then every person whose film was considered positive on *one or the other* of the two readings is referred for study.

If attitude (*2*) prevails, then only persons whose films were read positive by *both* readers are referred for study.

The objectives of attitude (*3*) may possibly be approached by having the films on which the two readers disagreed studied further by a single reviewer or by a panel of experts.

Once the attitude is established, it is possible to determine from the material of the present paper the gain in "truly positives" and the penalty in terms of false positives which will result from the method adopted.

In order to facilitate such evaluation, the different methods of utilizing dual reading are summarized in table 9.

This table contrasts the yield in "truly positives" and in false positives of a single reading and of the different methods of dual reading in the present study. It may serve as a basis for reaching a decision as to the most suitable method of dual reading and will also provide a quantitative estimate of the probable number of false positives and false negatives which will result from the decision adopted.

Table 9 was constructed on the basis of a hypothetical survey on a group which contains 100 "truly positive" cases. Under the conditions of the present study the group surveyed consists of 5,967 individuals. (All questionable cases are excluded.) If the contemplated survey is in a group which is much different from the one on which the present study is based, the numbers of false positives will be slightly different but the main results will be about the same.

It is seen in table 9 that a single reading will detect only 68 of the 100 "truly positives" and will give false positive diagnoses on 99 of the 5,867 "truly negatives." The largest number of "truly positives" detected (79 "truly positives") is provided by that method of dual reading in which every person whose film was diagnosed as positive by one *or* the other of the readings is examined. This method, however, selects also the largest number of false positives (192 false positives).

If one method had to be selected which would be of most practical use, the method of choice would seem to be that which utilized a single reader for reviewing all films in which the two readers were in disagreement. In other words, all films which were diagnosed as positive by *both* readers are immediately referred as positive. The films with contradictory diagnoses are reviewed by a competent reader and only those which he considers positive are referred for further study. It is, however, important that the reviewer be selected with great care. Test material and methods need to be devised which will select reviewers best qualified for the task.

From the example of the more successful reviewers in the present panel, it

may be estimated that such a procedure would detect 78 of the "truly positives." This is equivalent to 15 per cent more than are detected by a single reader, or to 30 per cent of the "truly positives" missed by a single reader. This is accomplished without any penalty since the number of false positives selected by this method is the same as that selected by a single reader, which was 99.

The following points of interest may also be seen in table 9.

TABLE 9

Summary Table: Comparison of Single Reading and the Different Methods of Dual Reading

Number of "truly positives"* detected and number of "truly negatives"* diagnosed falsely as positive in a *hypothetical* 70-millimeter survey of a group of 5,967 persons. This is an extrapolation from a real group of 1,790 persons presenting 30 "truly positive"* films to a group sufficiently large to present an even 100 "truly positives"* (exclusive of questionable films).

METHOD OF READING		SURVEY OF 5,967 PERSONS CONTAINING			
		100 "truly Positives"*		5,867 "truly negatives"*	
				falsely diagnosed as positive	
		Number of "truly positives"* detected	Number of "truly positives"* missed	Number	Per cent (c/5,867) × 100
		a	b	c	d
Single reader		68	32	99	1.7
Two readers (one *or* the other)		79	21	192	3.3
Two readers (*both* agreed)		57	43	6	0.1
Panel of three; two readers in agreement plus review of disagreements when the decision of the panel was	Positive by unanimous opinion	75	25	72	1.2
	Positive by at least one reader	78	22	107	1.8
"Panel" of one; two readers in agreement plus review of disagreements by a single reviewer	Average of five performers	74	26	83	1.4
	Average of two best performers	78	22	99	1.7

* For definition of "truly positive" and "truly negative" see text.

First it is important to note that films which are diagnosed as positive by *both* readers contain a negligible number of false positives. Therefore, no matter what scheme of dual readings is adopted, persons who are diagnosed as positive on two independent readings should immediately be referred for further intensive study. This information can also be utilized in communities where facilities are limited. If a system of priorities needs to be established, this group should have first call on the facilities of the community.

It should also be noted that a single reader selects only 67.8 per cent of the

"truly positives." Consequently, all the estimates on the prevalence of tuberculosis which are based on single readings are gross underestimates. The more nearly correct figure may be obtained by increasing the estimates by 50 per cent.

It is necessary to call attention to the fact that the study is based on a relatively small number of positive cases. In order to obtain more stable figures, it would have been necessary to study more than 5,000 survey films rather than the 1,800. This, however, was not possible because of lack of funds.

Because of the small numbers involved, the actual percentages shown are subject to considerable variation, but the relative comparisons between the different methods are valid. Additional confidence may be attached to the figures because of the multiplication of readings. It would, however, be desirable for other workers to repeat this experiment.

SUMMARY

In this study an attempt was made to evaluate the usefulness of dual reading in mass radiography. The study is based on 1,807 photofluorograms which were interpreted independently ten times. The following findings are recorded:

1. The subjective errors in film interpretation were of disturbing magnitude. The number of positives found by six readers varied from a low of 38 to a high of 83.

2. A single reader missed, on the average, 32.2 per cent of the films with evidence of inflammatory disease requiring clinical follow-up ("truly positive" films), and gave false positive diagnoses on 1.7 per cent of the "truly negative" films.

3. Films called positive on *one or the other* of two independent readings contained one-sixth as many more "truly positives" as were detected by a single reading. This is equivalent to 34 per cent of films missed by a single reading. However, the number of false positives was nearly doubled.

4. Films called positive on *both* of two independent readings contained only a negligible number of false positives, but the number of "truly positives" detected on both readings was less by one-sixth than that detected by a single reading.

5. Nearly the same results may be anticipated when the second reading is performed by the same reader as when two different readers interpret the survey films, provided the two readings are completely *independent*.

6. A panel reviewing the survey films with contradictory diagnoses succeeded in retaining a large proportion of the "truly positives" and in rejecting a considerable number of false positives. A balanced procedure may therefore be one which refers all films called positive by *both* readers and submits those with contradictory diagnoses to panel review.

7. A single competent reviewer may accomplish as good results as those achieved by a panel of three. Either procedure will detect 30 per cent of "truly positives" which are missed by a single reader, with no increase in the number of false positives.

8. In table 9 the performance which may be expected from the different

methods of dual reading is summarized. It provides data on which a selection may be made of the method most suitable for a given community.

SUMARIO

El Papel de la Lectura Doble en la Radiografía a Colectiva

En este estudio tratóse de justipreciar la utilidad de las lecturas dobles en la radiografía en masa, tomando como base 1,807 fotorroentgenografías que fueron interpretadas independientemente diez veces. Preséntanse los siguientes hallazgos:

1. Los errores subjetivos en la interpretación de las radiografías alcanzan una magnitud alarmante. El número de positivas observadas por distintos lectores varió de un mínimo de 38 a un máximo de 82.

2. Un solo lector pasa por alto un promedio de 32.8 por ciento de las películas con signos de enfermedad inflamatoria que exigen observación subsiguiente (radiografías "verdaderamente positivas"), y comunica diagnósticos "seudopositivos" en 1.7 por ciento de las "verdaderamente negativas."

3. Las películas clasificadas como positivas en *una u otra* de dos lecturas independientes contienen un sexto más de "verdaderamente positivas" que las descubiertas en una sola lectura, lo cual representa 35 por ciento de radiografías por alto en una sola lectura. Aun así, el número de "seudopositivas" casi dobla.

4. Las películas declaradas positivas en *ambas* de dos lecturas independientes sólo contienen un número menospreciable de "seudopositivas," pero el número de "verdaderas positivas" descubiertas en ambas lecturas es menor en un sexto que el descubierto en una sola lectura.

5. Cabe esperar más o menos el mismo resultado cuando ejecuta la segunda lectura el mismo lector que cuando dos lectores distintos interpretan las películas de la encuesta, con tal que las dos lecturas sean absolutamente *independientes*.

6. Un jurado que repase las radiografías de la encuesta en que hay diagnósticos contradictorios logra retener una gran proporción de las "positivas verdaderas" y rechazar un número considerable de las "seudopositivas." Parece, pues, lógico aceptar todas las radiografías declaradas positivas por *ambos* lectores y pasar al jurado de repaso aquellas en que existan diagnósticos contradictorios.

7. Un solo repasador competente puede obtener resultados tan buenos como los conseguidos por un jurado de tres personas. Uno u otro procedimiento descubrirá 28 por ciento de las "positivas verdaderas" que pasa por alto un solo lector, sin aumento en el número de "seudopositivas."

8. En la Tabla 9 del texto inglés sumarízase lo que cabe esperar de los diversos métodos de lectura doble, aportándose allí datos en que puede basarse la selección del método más apropiado para una colectividad dada.

Acknowledgment

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films with contradictory diagnoses. Doctors Elizabeth S. Heald and Merrell Sisson assisted in conference evaluation and panel review. Mr. Joseph E. Wineland, head technician of Cowell Hospital, was extremely helpful in managing the technical aspects of the study. Miss Elinor Crocker and Mrs. Lee Herrera assisted in the statistical work. Dr. R. R. Newell read the manuscript and offered valuable suggestions. The entire project was made possible by the cooperation of Dr. William G. Donald, University Physician.

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FRIEDLÄNDER'S PNEUMONIA[1,2]

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INTRODUCTION

With the advent of effective antimicrobial therapy, the clinical course of pulmonary infection has been markedly altered. In the instance of pneumococcus pneumonia the infection has been controlled so effectively that a complete cure without complication is the expected outcome.

In other infections such as tuberculosis and Friedländer's pneumonia, it has been possible to modify the course of the disease. In both these latter conditions the pathologic response is necrosis of lung parenchyma, and the bacterial infection is stubborn, with persistence of the offending microorganism in the diseased areas. Subsequent healing in the majority of cases is by fibrosis with a persistent anatomical defect such as cavitation or bronchiectasis. It is these pathologic responses and the obvious failure of any chemotherapeutic agent to affect tissue already necrotic, or to penetrate fibrosis of a well-established cavity, that are responsible for the limitations in the treatments of these infections.

CLINICAL MATERIAL

The 16 cases of Friedländer's pneumonia which are the basis of this report were cared for at Bellevue Hospital in the five-year period between 1944 and 1949. The infecting organism in all cases was reported as Friedländer's bacillus Type A. The incidence of Friedländer's pneumonia to all pneumonias has been variously reported to be between 0.6 and 4 per cent.

It is interesting to note that in the 19-year period between 1930 and 1949 only 6 patients with Friedländer's pneumonia were admitted to the Presbyterian Hospital in New York City. This divergence in the number of admissions of this disease in these two hospitals may be explained in part by the type of indigent patient found in a city hospital such as Bellevue.

Predisposing factors: In the present series of 16 patients the ages varied between 35 and 69 years with an average age of 52 years. One-half of the patients were severe alcoholics, and the ratio of males to females was 8:1. One was diabetic and one, syphilitic. These facts are in agreement with the observation of others that Friedländer's pneumonia is found for the most part in debilitated alcoholic men over 40 years of age.

What part oral hygiene and previous pulmonary infection play it is difficult to be certain. In this group, however, one patient had a long-standing bronchiectasis; another had silicosis, pulmonary fibrosis, and emphysema; and a third had had a pneumococcus pneumonia in the same lobe the previous year.

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[2] Presented in part before the Eastern Section of the American Trudeau Society at Saranac Lake on October 8, 1949.

Clinical manifestations: The majority of the patients were admitted to the hospital with a massive lobar consolidation and gave a history of cough, malaise, chills, and often chest pain of four to ten days' duration. The degree of prostration and cyanosis was marked. Although in 4 the sputum was the classical sticky viscid type, in others it was not, and in several instances it was "prune juice" in character. The roentgenogram in this stage showed the distended consolidation of a single lobe or, in 2 cases, of two lobes.

Those who were admitted with a longer history, ranging from two to six weeks, had less prostration and invariably had abscess formation. Even in the case of the patient who died six days after the onset of symptoms, at autopsy the typical large soggy lobe contained multiple abscesses. The necrosis of lung tissue occurs early and in most cases is extensive. In 4 of the patients who required thoracotomy drainage of abscess in the acute phase, large sloughs of necrotic lung could be picked out of the abscess cavities. Taylor (1) and Humphreys (2) have separately reported complete lobectomy by this removal of a slough in Friedländer's abscess of the lung. In 5 cases in the present series empyema was present, and in 2 of these the lung ruptured into the pleura to produce a pyopneumothorax.

Pathologic changes: The pathologic changes encountered in the disease after subsidence of the acute process, were demonstrated at operation by removal of the affected lung tissue in 10 cases. When there was persistence of cough and sputum, often associated with recurrence of febrile episodes and exacerbations of suppurative pneumonia, the lung was markedly fibrotic with multiple abscesses and a densely adherent and thickened pleura. There was extensive lymph node enlargement in the hilus, with dense matting of the vessels to adjacent structures. In many instances the pulmonary vessels were more friable than usual.

It is noteworthy that the right upper lobe was involved in one-half of the cases and that the upper lobes were the site of infection in 11 of the 16 patients.

THERAPY

Antimicrobial therapy: The experience with Friedländer's pneumonia at Bellevue Hospital from 1920 to 1935 was reported by Solomon (3) in 1937 and Hyde and Hyde (4), in 1942, published a report including cases from the New York Hospital as well. Since Solomon's series fell in the era before sulfonamide therapy and the Hydes' series was in the period before the introduction of streptomycin, the two series afford a basis for a rough comparison with the present therapeutic measures (table 1). Moreover, at the time of both of these previous studies, drainage of a chronic abscess or empyema was performed only occasionally. In the present series, 10 of the 16 patients had either lobectomy or pneumonectomy for residual disease. In the Hydes' report, the problem of the chronic infection was recognized and remarked upon as follows: "The ideal management of the non-healing residual cavity is not clear as there have been too few cases." Now the problem is more evident because the patients survive the acute stage of the disease to enter the chronic phase with residual abscess cavity and infection.

The use of antimicrobial agents is, in the great part, responsible for the marked drop in the case mortality of Friedländer's pneumonia. In table 2 the various agents used in the present study are tabulated. It is obvious that no single drug of those employed is unquestionably specific. Streptomycin was the drug of choice and is probably more effective than sulfadiazine. Preliminary trials using chloramphenicol (Chloromycetin), however, suggest that this may supplant both drugs as the preferred agent. It may become necessary to use several antimicrobial agents simultaneously in order to provide the most effective therapy

TABLE 1

Case Mortality in Friedländer's Pneumonia

DATE	AUTHOR	NUMBER OF CASES	DEATHS	CASE MORTALITY
				per cent
1937	Solomon (3) (Acute)	32	31	77.2 {97
1940	Solomon (5) (Chronic)	12	3	{23.5
1937	Bullowa (6)	41	34	83
1943	Hyde and Hyde (4)	51	26	51
1949	Present series	16	3	18.7

TABLE 2

Antimicrobiol Therapy in Friedländer's Pneumonia

	NUMBER OF CASES	DEATHS
Sulfadiazine	3	1 postoperative
Sulfadiazine and penicillin	3	
Penicillin alone	1	1
Streptomycin, sulfadiazine, and penicillin	3	1 postoperative
Streptomycin and sulfadiazine	3	
Streptomycin and penicillin	2	
Total	16	3

against secondary invaders, such as *Streptococcus*, which may complicate a pneumonia originally initiated solely by Friedländer's bacillus.

Surgical therapy: In addition to the role of antimicrobial therapy in the control of the acute stage, surgical drainage of the resulting abscesses, or drainage of an associated empyema, has been an important factor. Furthermore, after the acute process has subsided, the resulting chronic suppurative pneumonia with cavitations, bronchiectasis, and fibrosis have made lobectomy or pneumonectomy imperative for cure in most instances.

In table 3 are listed the surgical procedures which were performed in 11 of these 16 patients. In all but 3 instances, it was necessary to perform a thoracotomy for drainage of abscess during the acute stage of the process. Subsequent surgery during the chronic stage of the disease entailed pneumonectomy

or lobectomy for removal of the fibrotic, cavitary, and bronchiectatic suppurative lung tissue in all but one case. In this instance, the residual chronic abscess cavity and bronchial fistula were closed by means of a muscle-plasty procedure.

The indication for pulmonary resection in these patients was the presence of chronic suppurative disease, often with persisting bronchocutaneous fistula following the drainage in the acute stage. All of these patients had persistent cough and sputum with residual cavities demonstrable on the conventional chest roentgenogram or by bronchography. The sputum in some instances continued to reveal Friedländer's bacillus on culture and this was considered a further indication for resection. Usually there was an interval of four to six months between the acute pneumonia and the performance of the definitive surgical excision of suppurative lobe or lung.

An investigation of the cause of death in the 2 cases in which fatality occurred following surgery reveals evidence of the chronicity of the Friedländer's bacillus infection. In both instances the cause of death was a massive postoperative pneumonia due to Friedländer's bacillus on the contralateral side. In one patient

TABLE 3

Surgical Procedures in 11 Cases of Friedländer's Infection of Lungs

PROCEDURE	NUMBER OF CASES	DEATHS
Drainage followed by pneumonectomy	2	1
Drainage followed by lobectomy	5	1
Drainage followed by muscle-plasty	1	
Lobectomy without drainage	3	
Total	11	2

the original acute Friedländer's pneumonia had occurred five years previously. In the interval the patient had had a persistent bronchocutaneous fistula and chronic cough productive of sputum positive for Friedländer's bacillus. This patient died six days after pneumonectomy. In the other fatal case a lobectomy was performed four months after the acute pneumonia and abscess drainage. Autopsy in both instances revealed a typical Friedländer's pneumonia from which the organism was grown.

One of these patients received sulfadiazine and the other received streptomycin before and after the surgical procedure. As, in both instances, one or the other of these drugs had been employed extensively during the acute course of the disease, it seems likely that the microorganisms were no longer drug-sensitive as demonstrated by the lethal spread of the infection under therapy with the corresponding drug.

Course of infection without surgical therapy: It is of interest to review the patients who did not come to surgery. They were 5 in number and are listed in table 4.

The first, M. G., had a fulminating pneumonia and died three days after admission. The second patient, J. M., is of special interest. In 1943 he had a

right middle lobe Friedländer's pneumonia from which he recovered and was discharged from the hospital. The following year (1944) he was readmitted with a Friedländer's pneumonia involving the left lower lobe, from which he again recovered, with a persistence of a large abscess cavity. Lobectomy was advised at this time but was refused. One year later (1945) he was again admitted after hemoptysis with sputum positive for tubercle bacilli. The large cavity was still present with tuberculous infiltrations throughout the lower lobe.

There were, in all, 3 instances in which tuberculosis was associated with Friedländer's pneumonia. The first has been described immediately above. The second patient developed sputum positive for tubercle bacilli while awaiting surgery for chronic suppurative pneumonia of the right upper and middle lobes. A bi-lobectomy was performed. In the third case, fibrocaseous tuberculosis was found in the removed lung after pneumonectomy, although the diagnosis had not

TABLE 4

Cases of Friedländer's Pneumonia Not Treated Surgically

PATIENT	NATURE OF LESION	TREATMENT	RESULT
M. G.	L.U.L. pneumonia	Penicillin	Died three days after admission
J. M.	1943 R.M.L. pneumonia 1944 L.L.L. pneumonia with abscess 1945 Hempotysis, tuberculosis, cavity persists with spread	Sulphadiazine Sulphadiazine	Transferred for continued tuberculosis care
J. W.	R. U. L. Pneumonia	Penicillin and sulfadiazine	Discharged with cavity R.U.L.
W. M.	L. U. L. pneumonia	Penicillin, sulfadiazine and streptomycin	Cavity L.U.L., signed out A.O.R.
J. W.	Bilateral patchy pneumonia with fibrosis	Penicillin and sulfadiazine	Pneumonia cleared, diffuse fibrosis

been suspected before operation. The most likely explanation of the occurrence of tuberculosis in these 3 cases is invasion and activation of an old quiescent lesion by the suppurative pneumonia.

The third and fourth patients, J. W. and W. M., who did not come to surgery, both left the hospital with cavitary defects in the lungs. One left the hospital against advice and has not been heard from since; the other was a nurse who had a small residual cavity without symptoms and surgery was deemed unnecessary.

J. Z., the last patient, is interesting in that he is the only patient in this series who did not have a frank lobar pneumonia. He was admitted in an acute alcoholic state and was found to have a diffuse bilateral pneumonia with persistent discharge of Friedländer's bacillus in the sputum. He had an underlying silicosis, fibrosis, and emphysema but never developed demonstrable abscess.

Thus, although the immediate case mortality of Friedländer's pneumonia has been greatly reduced by antimicrobial therapy, it is also true that the disease

process in the great majority of cases is finally cured only by excision of the affected portion of the lung.

Four representative cases of this series are presented to illustrate the types of surgical procedures required.

CASE REPORTS

Case 1 (figure 1). W. McD., a 36-year-old alcoholic male, was admitted in 1945 with right middle lobe pneumococcus pneumonia. He was treated with sulfadiazine with complete recovery. He was readmitted one year later with a Friedländer's pneumonia of the same lobe, with sputum and blood cultures positive for this organism. He was treated with streptomycin, sulfadiazine, and penicillin. An abscess of the right middle lobe resulted, but did not require drainage. Right middle lobe lobectomy was performed six months later for persistent cough, sputum, and recurrent bouts of fever.

Case 2 (figure 2). M. Q., a 43-year-old alcoholic male, was admitted with right lower lobe Friedländer's pneumonia, which was treated with streptomycin and penicillin. He developed a lung abscess and empyema. Drainage was performed, with persistent bronchocutaneous fistula. This was closed by means of a muscle-plasty operation. Bronchiectasis is still present in the right lower lobe, but he has no symptoms. Excision of the bronchiectatic lobe has been deferred in this case because of lack of symptoms and minimal anatomical defect.

Case 3 (figure 3). J. E., a 49-year-old man with right upper lobe Friedländer's pneumonia, was treated with streptomycin. He developed a pyopneumothorax which required thoracotomy drainage. Muscle-plasty closure for persistent bronchocutaneous fistula was performed. Finally a right upper lobe lobectomy for residual cavity with cough and sputum was carried out one and one-fourth years later.

Case 4 (figure 4). P. L., a 42-year-old alcoholic male, was admitted with right upper lobe Friedländer's pneumonia and abscess. He received streptomycin and penicillin. Five months later lobectomy was performed for contracted lobe with abscess. Sputum was still positive for Friedländer's bacillus.

In all 4 of these patients, follow-up observations have been made for one to three years after recovery and the patients are symptom free.

SUMMARY

Experience in the treatment of 16 cases of Friedländer's pneumonia observed at Bellevue Hospital during the five-year period between 1944 and 1949 has been reviewed. A marked decrease in the case mortality rate, due to the use of antimicrobial and surgical therapy, was noted.

With the more frequent survival of patients in the acute phase of Friedländer's pneumonia, the problem of the residual chronic suppurative pneumonia is assuming much greater importance, and requires lobectomy or pneumonectomy for cure in a large precentage of cases.

There is some risk of reactivation of the Friedländer's infection postoperatively even though surgery be performed as long as five years after the original acute pneumonia. Consequently, it is advisable to ascertain the *in vitro* drug resistance

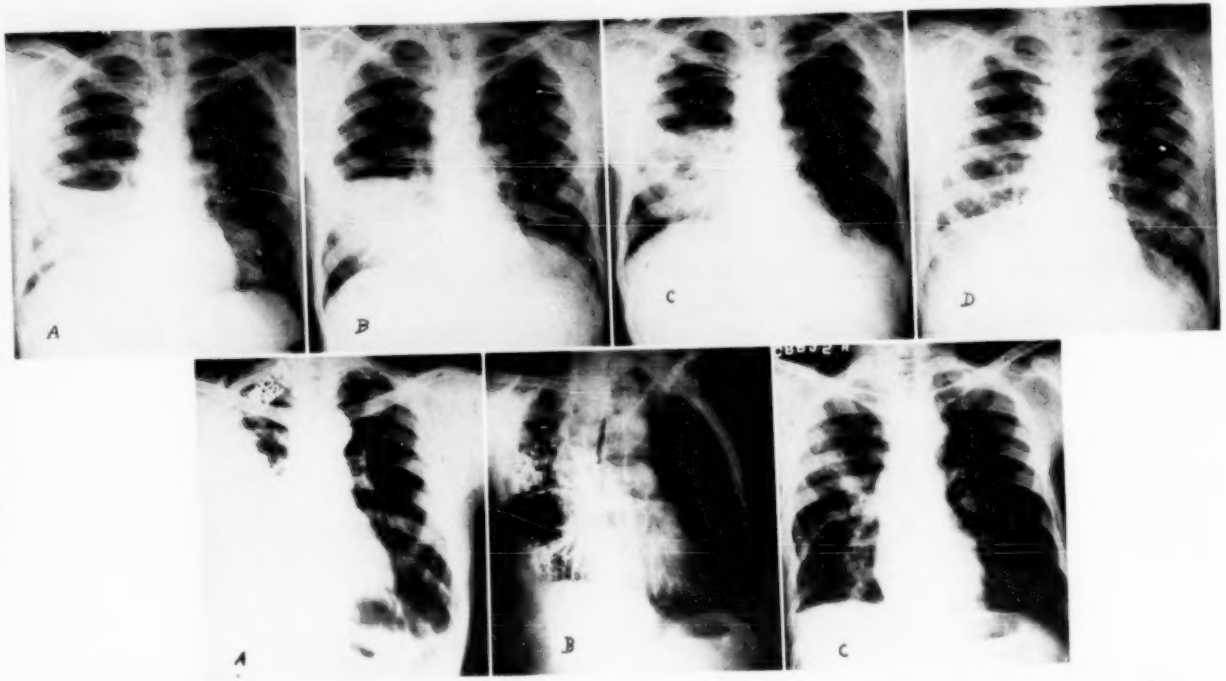

FIG. 1 (Case 1) (Top) A. Pneumococcus pneumonia involving middle lobe. B. Friedländer's pneumonia of same lobe one year later. C. Residual abscess following Friedländer's pneumonia. D. Roentgenogram after lobectomy of right middle lobe.

FIG. 2. (Case 2) (Bottom) A. Right lower lobe Friedländer's pneumonia. B. Bronchogram prior to muscle-plasty closure showing bronchiectasis in lower lobe five months later. C. Roentgenogram one year following closure of bronchocutaneous fistula by muscle plasty.

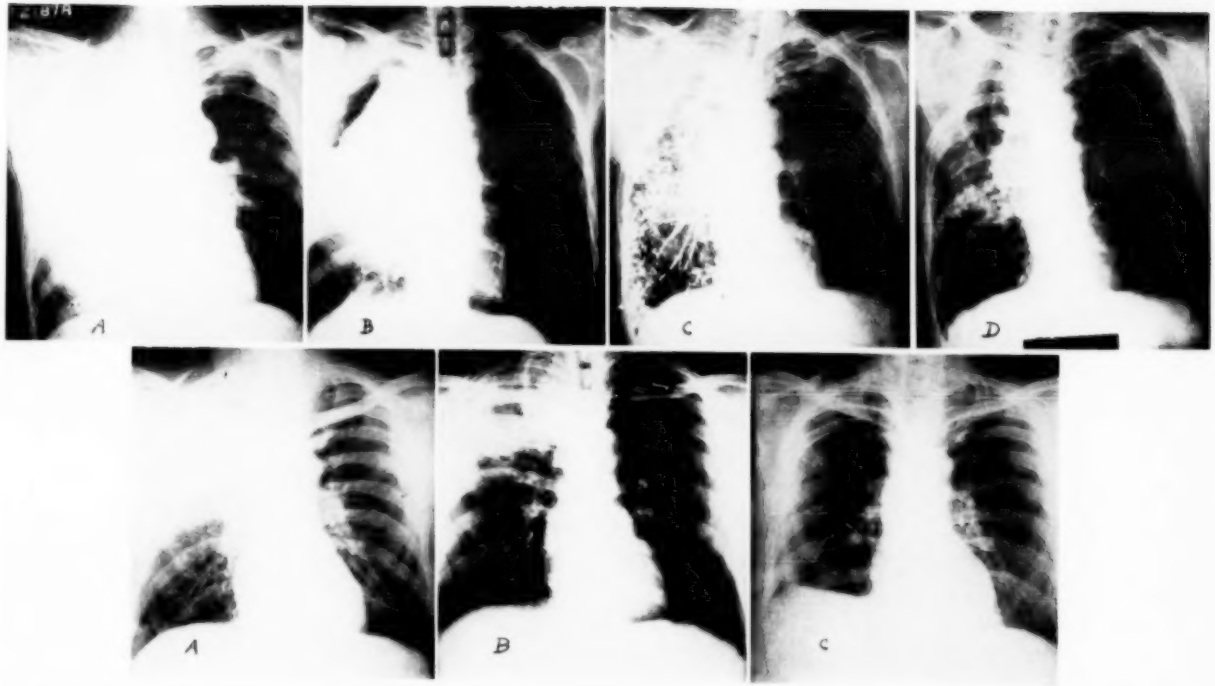

FIG. 3. (Case 3) (Top) A. Right upper lobe Friedländer's pneumonia. B. Spontaneous pyopneumothorax which required drainage. C. Residual cavity by bronchogram following muscle plasty closure of bronchocutaneous fistula four months later. D. Roentgenogram one year and three months after right upper lobe lobectomy.

FIG. 4. (Case 4) (Bottom) A. Right upper lobe Friedländer's pneumonia with abscess. B. Contracted right upper lobe with persistent cavity three months later. C. Roentgenogram following right upper lobe lobectomy, one month after B.

of the Friedländer's strain harbored by the patient before operation so that the most effective combination of drugs for chemoprophylaxis can be chosen.

SUMARIO

Neumonía de Friedländer

Repásase lo observado en 16 casos de neumonía de Friedländer, tratados en el Hospital Bellevue durante el quinquenio entre 1944 y 1949.

La serie muestra una pronunciada baja en la mortalidad, debido al empleo de la quimioterapia, los antibióticos y la cirugía.

Una vez sobrevivida la fase aguda de la neumonía de Friedländer, el problema planteado por la fase supurativa, crónica, residual exigió la lobectomía o la neumonectomía para la curación en un porcentaje elevado de los casos.

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ACUTE LUNG ABSCESS[1,2]

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This report on acute lung abscess is based on our experience with 35 consecutive patients seen at this hospital during the past three years (June 1946 to April 1949) in whom a diagnosis was made on the basis of physical, clinical, and radiologic findings. The study of this group of patients was undertaken because it was believed that there was a need for re-evaluation of our results and methods in the treatment of lung abscess in the light of newer therapeutic agents.

MATERIAL

All of the 35 patients reviewed were males, and their ages varied from 31 to 70 years with a mean age of 47 years. The probable etiology of the abscesses as deduced from the history and clinical findings is as follows:

1. Bronchial embolism (3) was believed to be the causative agent in 17 patients. Nine of these occurred in postoperative patients; 3 were associated with alcoholic debauches; and 3 were associated with contralateral pulmonary pathology (contralateral pneumonia in one and bronchiectasis in 2).

2. Two abscesses apparently developed subsequent to pulmonary infarcts, both occurring in postoperative patients with evidence of peripheral venous disease.

3. In 14 patients the abscess followed a progressive suppurative pneumonitis.

4. In 2 patients acute abscess developed in the course of bronchiogenic carcinoma, and will not be included in the discussion of this series.

Bacteriologic studies: The bacteriologic studies of the sputum and bronchial secretions of these patients for the most part revealed a mixed bacterial flora. In none of the patients were acid-fast bacilli demonstrated on smear or by cultural methods. The most common organisms found were beta hemolytic *Streptococci* and *Staphylococci* and these were found in combination in 23 patients. *Streptococcus viridans* was the sole organism isolated in 4 cases. In 4 patients *B. pyocyaneus* was isolated as the only organism. *H. influenzae* was found in one patient in association with streptococci and *Microphiles aeruginosa*. Fusiform bacilli and Friedländer's bacilli were each isolated from one case, but in each case were associated with other bacteria. *E. Coli* was isolated in 2 cases, however, in which no other organisms were demonstrated.

Clinical manifestations: The primary symptoms presented by these patients

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[2] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

were cough, expectoration, chest pain, chills and fever, hemoptysis, and foul sputum. All of the patients experienced cough and expectoration. Thirty patients noted chest pain, usually localized quite well to the site of the lesion. Chills and fever were noted by 21 and hemoptysis and foul sputum were noted by 19 and 14 patients, respectively.

The duration of the disease from the onset of symptoms to the time of diagnosis varied from three days to two months. It will be noted that 2 patients are included in this series whose symptoms had existed longer than the six-week period established arbitrarily by Neuhof and Touroff (9, 10, 11) as the time interval at which an abscess should be considered chronic. It is believed that these cases belonged in the group of acute cases, however, because at the time a diagnosis was made the patients presented acute symptoms, signs, and radiologic findings of progressive disease.

The location of the pulmonary lesion was in the right upper lobe in 12 patients (right upper and middle lobe in one); right lower lobe in 12 patients; left lower lobe in 6 patients; and left upper lobe in 5.

Diagnosis: In arriving at a diagnosis, the history and physical findings were usually suggestive, but the radiologic findings and bronchoscopic appearances were considered the two most helpful diagnostic aids. Roentgenographic evidence of infiltration and/or cavity formation were noted in all cases. There was also bronchoscopic evidence of acute endobronchial inflammation in or around the involved segmental bronchus in all patients. In 21 patients (60 per cent), foul bronchial secretions were noted at bronchoscopy.

RESULTS OF THERAPY

A consideration of the therapy given this group of patients must be prefaced by an explanation that they have been constantly followed by a combined medical and surgical chest service staff which we believe is a most important factor in the proper evaluation and handling of this type of pulmonary disease. In addition to daily rounds made by the resident and consultation staff, each of these patients was presented weekly to a combined conference of the medical and surgical services. At this conference, after reviewing the patient's clinical course and recent roentgenograms, a decision was made to continue or discontinue the therapy being used and/or to institute some surgical procedure. Each patient received parenteral penicillin in divided doses varying from 240,000 to 1,000,000 units daily, after a diagnosis had been made. In addition aerosol penicillin, in dosages of 20,000 to 80,000 units every four hours, was given by means of an oxygen pressure-nebulizer with 1:5,000 aqueous zephiran as the usual vehicle.

As supplementary therapy, 15 patients received sulfadiazine orally; aerosol streptomycin was given to 10 patients and parenteral streptomycin was given to 2 patients; postural drainage was utilized and supervised in 31 patients. Thirty of these patients received at least one blood transfusion consisting of 250 to 500 cc. of citrated whole blood.

Surgical therapy was utilized in 9 patients because of the progression or per-

sistence of the lesion in spite of what was believed to be adequate therapy. Drainage of the abscess as a one-stage procedure was performed in 4 patients; in all of these cases the abscesses were close to the chest wall, large, easily accessible for drainage, and the patients were desperately ill. Drainage followed by secondary decortication and segmental resection was performed in one patient in whom a moderate-sized putrid empyema, which had developed as the patient was being observed in the hospital, was encountered at the time of drainage. Exploratory thoracotomy with external drainage was utilized in 2 patients. In these patients bronchogenic carcinoma was strongly suspected preoperatively so that anterior explorations were carried out which did not reveal tumor, and posterior drainage of the abscesses was performed. These patients were 57 and 61 years of age, respectively, and they recovered promptly from these procedures. Lobectomy was performed in 2 patients in whom central cavities in the right lower lobe along the mediastinal surface were present and clinical improvement had reached a plateau. Three of the patients in whom only drainage of the abscess was performed had later closure of the cavity by musculoplastic operations.

It is evident that in this group of 33 patients, 26 were treated solely by a medical regimen. In discussing the results the series has been divided into groups of those patients who required surgical therapy and those who did not, merely for ease of presentation. It is not meant to imply by this division that these two groups of cases are comparable, because the surgically treated cases were obviously those which did not respond favorably to an adequate medical regimen.

In the 9 patients treated surgically, no operative deaths occurred. One of the patients with bronchogenic carcinoma died three months following drainage of an acute abscess, the cause of death being extensive metastases from the inoperable carcinoma which had caused the abscess. The 8 remaining patients are well, without symptoms, and have shown either complete clearing or minimal scarring in the region of the previous lesion.

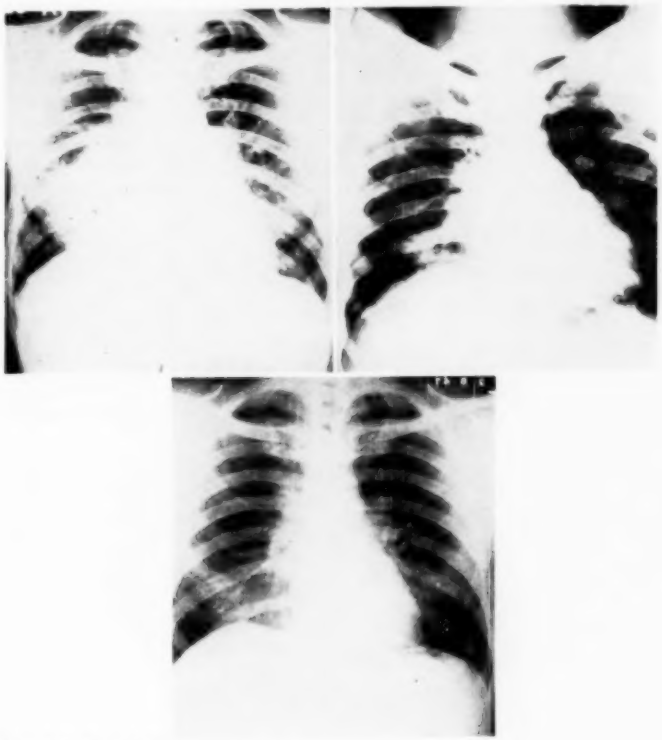
Of the 26 patients treated by medical regimen alone, all have recovered, as manifested by complete clearing of the roentgenographic evidence of the lesion. Seventeen of these patients are apparently well and have been without symptoms for more than a year following radiologic disappearance of their lesions Six of the medically treated group have continued to have symptoms in spite of radiologic clearing of the lesion. Two of these patients complain of a dry cough, but repeated thoracic roentgenograms and bronchograms have revealed no abnormalities. Three patients have been shown to have extensive bronchiectasis as a cause for their symptoms of cough and expectoration. One patient has continued to have intermittent streaking hemoptysis, although radiologic studies, bronchograms, and repeated bronchoscopies have revealed no abnormalities.

Two patients in this group have died. Post-mortem studies in both of these cases revealed the cause of death to be coronary infarction, and examination of the previous area of abscess showed complete healing of the lesion.

The following cases are cited as being illustrative of the groups which have been described:

Case Reports

Case 1. J. T. S. (No. 92826), white, married, 36-year-old male, was admitted to this hospital on December 18, 1946 with complaints of cough, expectoration, fever, and hemoptysis which followed a tonsillectomy under local anesthesia two months previously. The patient's



FIGURES 1-3. Case 1.

FIG. 1. (Upper left) Roentgenogram of thorax on admission showing a large area of infiltration in the right lower, central lung field with an area of cavitation which was evident on the original films.

FIG. 2. (Upper right) Thoracic roentgenogram nine days after admission showing clearing of the pneumonitis but persistence of the cavitation.

FIG. 3. (Bottom) Roentgenogram prior to discharge showing complete radiologic clearing of the previous lesion in the right lower lung field.

symptoms had been progressive in spite of bed rest and therapy with sulfadiazine and parenteral penicillin given in adequate therapeutic dosages.

Examination on admission revealed a well-developed, obviously ill male. Chest examination showed coarse rhonchi over the right chest. There were roentgenographic findings consistent with a cavity and surrounding pneumonitis in the central right lung field (figure 1). Clinical studies revealed a hemoglobin concentration of 12 Gm. per 100 cc. The erythro-

cyte count was 3,750,000 per cu. mm. The total leukocyte count was 22,000 per cu. mm. with a differential count of 80 per cent segmented polymorphonuclear leukocytes. The urinalysis was normal.

Bronchoscopic examination showed a marked inflammatory reaction with ulceration in the right intermediate and lower lobe bronchi; there was also marked retention of purulent, foul secretions. The patient's temperature varied from 98°F. to 103°F. daily.

Bacteriologic examinations of the patient's sputum and bronchial secretions yielded the following results. Twelve smears examined for acid-fast bacilli and 9 cultures for tubercle bacilli were negative. A multiplicity of organisms were isolated from the sputum and bronchial secretions, including *Staphylococci*, anaerobic *Streptococci* and *Micrococci*.

After admission the patient was placed on a regimen of postural drainage; penicillin was given parenterally and by aerosolization and the patient received four transfusions of 250 cc. of whole blood. His course was one of gradual but definite improvement, which the patient and his physicians stated was particularly notable after beginning aerosol therapy. Nine days after admission, roentgenographic examination showed a decrease in the previously noted pneumonitis although the cavity could still be seen (figure 2). On December 30, 1946 the patient's temperature returned to 98.6°F. and remained there during the remainder of his hospital stay. The total leukocyte count at this time was 9,600 per cu. mm. On February 8, 1947 there was radiographic evidence (figure 3) of apparent complete clearing of the previous abscess. The patient was asymptomatic, and he was discharged from the hospital on March 14, 1947. Follow-up examinations at three-month intervals have shown no changes in the roentgenograms of the chest and the patient has remained asymptomatic. Bronchograms performed in November 1947 revealed a normal bronchial tree with no evidence of bronchiectasis and a follow-up bronchoscopy revealed a normal tracheobronchial tree.

Case 2. G. L. C. (No. 92761), a 31-year-old, married male, was admitted to this hospital on December 14, 1946 with complaints of cough, foul sputum, hemoptysis, fever, chills, weakness, anorexia, and a 35-pound weight loss of one month's duration.

This patient gave a history of having had a tonsillectomy performed under local anesthesia one month prior to admission. Following this procedure he developed a cough which gradually became productive of one-half to one cup of foul, yellow sputum daily. These developments were associated with recurring bouts of chills, fever, night sweats, increasing weakness, fatigue, and anorexia, and he rapidly lost 35 pounds. This chain of events developed progressively in spite of therapy with sulfadiazine and penicillin in adequate amounts.

Physical examination showed an ill-appearing male with a temperature of 102°F. and evidence of cough, foul sputum, and weight loss. Examination of the chest revealed coarse rales in the right chest. The hemoglobin concentration was 11.5 Gm. per 100 cc. of blood; the erythrocytes numbered 3,560,000 and the leukocytes 12,000 cells per cu. mm. with a differential count of 68 per cent polymorphonuclear leukocytes, 24 per cent lymphocytes, 4 per cent eosinophils, and 4 per cent monocytes. A serologic test for syphilis was negative, and the hematocrit was 37 per cent.

Roentgenograms of the thorax showed an extensive penumonitis of the right central lung field (figure 4) which was found on further examination to involve the right lower lobe. Bacteriologic examination of the sputum and bronchial secretions revealed a mixed bacterial flora. Three smears showed no acid-fast bacilli and six cultures showed no growth of acid-fast organisms. *Staphylococci*, *Streptococci*, and *Monilia* were isolated on cultures from the sputum.

Bronchoscopy revealed a red, edematous, inflammatory reaction in the right intermediate bronchus, and a large quantity of foul secretions was aspirated from the right bronchus.

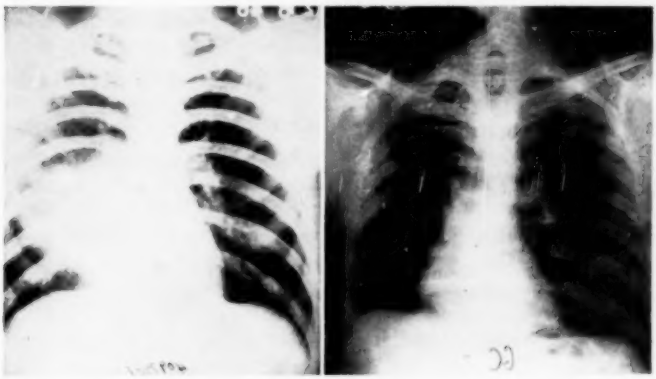
The patient was placed on a regimen of postural drainage and parenteral and aerosol penicillin, with a dramatic improvement in his cough, expectoration, chills, and fever. Two days after beginning aerosol penicillin his temperature was 98.6°F., and the patient noted a complete subsidence of foul sputum. Follow-up roentgenograms one week after

admission showed a clearing of the pneumonitis but there was a persistent cavitation in the right lower lobe.

In spite of this apparent improvement, however, the patient continued anorexic and he did not gain weight although he was eating a high caloric, high-vitamin, and high-protein diet. Two weeks after admission there was a return of some cough, expectoration, and some return of the pericavitary pneumonitis was seen on the roentgenogram, so that surgery was deemed imperative.

The patient was prepared for surgery by repeated small blood transfusions and a continuation of the parenteral and aerosol penicillin. On February 14, 1947 a right lower lobectomy was performed under general endotracheal anesthesia, using the technique of hilar dissection and individual treatment of the hilar structures. The excised lower lobe revealed an acute lung abscess with surrounding pneumonitis in the hilar portion of the lobe.

The patient's postoperative course was uneventful, but his return of appetite and rapid



FIGURES 4 and 5. Case 2.

FIG. 4. (Left) Roentgenogram of thorax on admission showing an extensive infiltration of the right central lung field. On the original films a central cavitation in this lesion was clearly apparent.

FIG. 5. (Right) Follow-up roentgenogram one year following lobectomy. Postoperative changes in the right chest and absence of the eighth rib are evident.

gain of weight were remarkable. In a period of two weeks postoperatively he had gained 30 pounds. He was discharged from the hospital March 7, 1947.

A follow-up examination on May 6, 1947 showed that the patient had gained more weight. He had gone from a preoperative weight of 118 pounds to 170 pounds; he had no complaints and he had been working as a truck driver for more than one month. Repeat bronchoscopy and follow-up bronchograms at this time revealed no abnormalities of the bronchial tree. The patient has remained well up to the present time and there has been no change in the roentgenograms since the follow-up films obtained in March 1948 (figure 5).

DISCUSSION

The most important advance in the treatment of lung abscess prior to 1944 was the introduction of the principle of early surgical drainage of the abscess through the chest wall by Neuhof and Touroff (9, 10, 11) and others (2, 4, 5,

7, 8, 13). By operating on patients between the fourth and tenth weeks these surgeons reduced the case fatality rate of this disease from about 40 to 10 per cent (1, 11). After the discovery of penicillin, however, a powerful antimicrobial agent was made available which has undoubtedly favorably affected the results in this disease.

Since study of these cases was begun, excellent papers on this subject have been published by Smith (14), Glover and Clagett (6), and Stivelman and Kavee (15) which have presented rather divergent views regarding the management of these lesions. We believe that this series of cases demonstrates the need for carefully and closely combining surgical and medical methods in treating patients with lung abscess, rather than presenting these methods as competing techniques.

It has been our impression that addition of aerosol penicillin to the armamentarium of therapy for this disease has been a most important factor in the recovery of the patients observed. This impression has been based upon the rapid clearing of acute endobronchial inflammatory reaction noted bronchoscopically, as well as the fact that some patients treated adequately with parenteral penicillin showed little or no clinical and radiologic clearing until they were given penicillin by the aerosol route.

In summarizing our final results in this group of cases, we have believed that the 2 patients with bronchiogenic carcinoma should be excluded from the recovery figures because the course of their progress was influenced by the carcinoma and not by the secondary abscess. This leaves a group then of 33 patients, all of whom recovered from acute lung abscess, an immediate recovery of 100 per cent. If one considers the 2 late deaths due to myocardial infarction, there was then an eventual recovery from acute abscess in 31 patients or 93 per cent. It is evident, however, that in the group of recovery patients there were 6 patients (18 per cent) who are obviously not well because of pulmonary disease. Three (9 per cent) of these patients have known bronchiectasis which may have antedated or occurred as the result of the abscess, and 3 patients (9 per cent) have pulmonary symptoms. All of these 6 patients may later require surgery for the sequelae of their original disease. In this small group of surgically treated cases, it is interesting that all of the patients have recovered and are well and without symptoms.

As mentioned above, bronchoscopy has been most useful as a means of diagnosis and localization in this group of cases. In individual cases, however, we have believed that the procedure was of definite therapeutic value in the removal of thick plugs of mucus and in dilating stenosed bronchi after subsidence of the acute inflammatory phase of reaction.

SUMMARY AND CONCLUSIONS

Thirty-five patients with acute lung abscess have been reported. Excluding 2 patients with bronchiogenic carcinoma, all of these patients have recovered from acute abscess with no mortality ascribable to the disease.

From a survey of these cases it is believed that the following conclusions may be drawn:

1. That lung abscess is as yet a not infrequent disease.
2. The consideration of a diagnosis of lung abscess demands continued cooperative care by physicians and surgeons until the patient is well.
3. Penicillin appears to be the most effective therapeutic agent at this time. In addition, it is believed that the drug should be given by aerosolization as well as parenterally.
4. Bronchoscopy is most important in the diagnosis and localization of the lesion, but it also has occasional value in therapy by removal of foreign material and in bronchial dilatations after subsidence of the acute phase.
5. Surgical therapy can be reserved for the patients who do not respond after a reasonable trial of therapy as outlined in this paper.
6. The patient who apparently recovers from an acute lung abscess should be carefully followed for an indefinite period following his recovery.

CONCLUSIONES

Absceso Pulmonar Agudo

Del estudio de los casos sumarizados, cabe sacar las siguientes conclusiones:

1. El absceso pulmonar es una enfermedad que aun no es rara.
2. La formulación de un diagnóstico de absceso pulmonar exige continua asistencia cooperativa de parte de médicos y cirujanos hasta que sane el enfermo.
3. La penicilina parece constituir nuestra terapéutica más eficaz por ahora. Además, parece que debe ser administrada por aerosolización así como parentéricamente.
4. La broncoscopia es de la mayor importancia en el diagnóstico y localización de la lesión, pero además posee de cuando en cuando valor en la terapéutica por retirar las materias extrañas y en las dilataciones bronquiales una vez pasada la fase aguda.
5. Puede reservarse la terapéutica cruenta para los enfermos que no responden al cabo de cierto tiempo razonable a la terapéutica bosquejada en este trabajo.
6. Todo enfermo aparentemente repuesto de un absceso pulmonar agudo debe ser mantenido en cuidadosa observación durante un período indefinido después de la reposición.
7. Los enfermos con absceso pulmonar agudo comunicados suman 35. Excluyendo 2 con carcinoma bronquiógeno, todos se han repuesto del absceso agudo sin mortalidad imputable a la enfermedad.

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THE RESISTANCE OF TUBERCLE BACILLI TO CHEMOTHERAPEUTIC AGENTS[1]

A Review of Basic Biological Considerations

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One of the most important obstacles to the use of streptomycin for the treatment of tuberculosis is the resistance of some tubercle bacilli to the action of the drug. This has created a need for an understanding of the mechanisms involved in the development of resistance. Although our present knowledge of the basic phenomena involved is limited, it may be useful to review certain facts and ideas which are important, and to try to reconcile some of the apparently conflicting views. No attempt will be made to make a detailed review of the general subject of resistance of microorganisms to chemotherapeutic agents, or to include a detailed discussion of the great mass of information which has accumulated concerning the chemotherapy of tuberculosis. Instead, this discussion is confined to a consideration of the pertinent literature related to the resistance of tubercle bacilli to streptomycin and other chemotherapeutic agents. In addition there will be included considerable unpublished experimental data from this laboratory on para-aminosalicylic acid, neomycin, streptomycin-dependent tubercle bacilli, and related subjects. Many suitable references were omitted because of the similarity of the material.

TERMINOLOGY AND GENERAL CONCEPTS

Before discussing these studies, it is desirable to make certain preliminary statements concerning terminology and the general concept of bacterial variation.

With the introduction of strict criteria for classification and of techniques for studying pure cultures of bacteria, the concept of monomorphism discouraged the study of problems dealing with bacterial variation and inheritance (1). Recently much attention has been given to the old observation that a bacterial population may contain variants which differ from the parent cells in a specific and permanent manner. These modifications have been attributed to various causative mechanisms: mutation, dauernmodifikation, dissociation, cyclogeny, adaptation, variation, et cetera (1, 2). The present disagreement as to the correct terminology to be employed when discussing the mechanism of resistance of tubercle bacilli to streptomycin is partly the result of failure to adhere to the definitions of the terms "adaptation" and "genetic variation." It should be emphasized that, by definition, adaptations or environmental modifications of parent organisms are not exhibited by progeny which have been removed from the environment that originally induced the changes in the parent. Thus, any modification which is transmitted from parent to progeny is not a result of

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

adaptation but is an inherited variation. Marked metabolic and morphological changes occur in bacterial populations as the result of adaptation (3, 4, 5, 6, 7). There are some who would ascribe bacterial resistance to antibiotics as an adaptation. Recent studies, reviewed so well by Luria (2) and Braun (8), have pointed out, however, that bacteria, like higher organisms, exhibit variations which conform to the requirements of genetic variation. Moreover, the study of bacterial variants resistant to chemotherapeutic agents has offered some of the most convincing evidence yet obtained that bacterial variations occur spontaneously, are undirected, and are inherited (2, 9, 10, 11, 12, 13, 14, 15).

In this report the word "mutation" is used, as defined by Luria (2), to mean "a permanent change affecting one or more properties of a bacterial cell and its offspring. The use of this term does not imply *a priori* identification with the process of gene mutation or with any type of hereditary change in higher organisms. Whatever similarities or differences exist should be discovered through study of specific cases."

There is now considerable evidence that bacterial variants resistant to chemotherapeutic agents arise as the result of mutations (2, 10, 14). Direct evidence that variants resistant to certain toxic salts may arise spontaneously in cultures obtained from *single cells* has been well presented by Severens and Tanner (16). Demerec (9, 10) and others have employed a simple technique (first proposed by Luria and Delbruck (17)) which demonstrates quite clearly that variants resistant to penicillin or streptomycin can originate from a few susceptible cells and that the resistance is not induced by the environment. From these studies it is now certain that an initially heterogeneous population is not required, but that single clones[2] from an originally sensitive cell may give rise to resistant mutants. The chemotherapeutic agent modifies the environment so that variants with selection value, in this instance drug-resistant cells, thrive. In brief, it has been repeatedly demonstrated that drug resistance is transmitted from parent to progeny in the absence of the drug, that the rate of mutation of resistant variants in the parent population is of the order of that found in other organisms, and that there is a fluctuation of the number of variants among different populations of the same bacteria. This fluctuation is what is expected with rare, undirected mutations (2, 17).

In 1927, Muller showed that irradiation with X-rays could increase the appearance rate of mutations. Since then numerous agents capable of influencing the mutation rate in a variety of organisms, including bacteria, have been discovered. At present, however, there is no definite evidence that the commoner antibacterial agents under discussion can act in such a manner as to increase the number of resistant variants.

The occurrence of bacterial variants is not a rare or unusual phenomenon, and variants resistant to the newer chemotherapeutic agents might have been anticipated before the drugs were actually employed. It is of interest that Erhlich described clearly the phenomenon of microbial resistance to chemo-

[2] Clone refers to an aggregate of individual cells, all of which are descended from a single cell.

therapeutics and laid down the principles for delaying or avoiding the development of resistance (18). Mutants differing from the parent cells with respect to a wide variety of specific characteristics have been studied. These include, for example, modifications involving color (19, 20, 21), virulence (22, 23), virus (phage) specificity (17, 24), metabolic processes (25, 26, 27, 28), et cetera. While certain bacterial modifications attributed to "training" are adaptations (29), many are true hereditary variations. Also, it is undoubtedly true that many so-called "pure strains" contain numerous variants which go undetected. In a relatively brief period of time, enormous numbers of cells may arise from an original sensitive parent cell and produce a significant number of variants resistant to the action of an antibacterial agent. It has been estimated (10) that the mutation rate of first-step penicillin-resistant *Staphylococci* is in the neighborhood of 1×10^{-8} and that the rates of mutation of a strain of *Escherichia coli* to streptomycin resistance and to streptomycin dependence are both approximately 1×10^{-10} per bacterium per division cycle (15). While these are low rates in an absolute sense, they become significant when related to large, rapidly multiplying populations of bacteria.

Because of the presence of variants in a bacterial population, the term "pure strain" is probably a misnomer if one assumes, as some do, that all cells in such a strain have identical characteristics. If the population is sufficiently large and if techniques for identifying specific modifications are available, it is likely that most of the cells will be similar but a few will be true variants. Cells of individual colonies derived from susceptible parent cells show marked variation in the degree of resistance to streptomycin. It has recently been demonstrated in this laboratory that the streptomycin resistance of individual cells of a susceptible strain of *Mycobacterium ranae* is distributed along a sigmoid curve, a cumulative probability curve (30). Also, so-called pure strains of streptomycin-dependent *E. coli* (31) and streptomycin-dependent *M. ranae* (32) vary greatly in their requirements for streptomycin. Thus, from a streptomycin-dependent strain of *M. ranae*, cells were obtained which grew best in the presence of 100 γ of streptomycin per ml. of medium, while other cells obtained by repeated subculturing of the same strain grew neither in the absence of streptomycin nor in the presence of more than 50 γ of the drug per ml. These examples demonstrate that there is not only absolute variation with relation to an individual drug but also relative variation.

The terms "parent population" or "parent cells" refer to a group of cells of the same strain, the vast majority of which have similar properties when they are cultured and examined under similar conditions. In this paper, these critical properties will generally refer to the susceptibility or resistance (or dependence for growth) of the cells to a particular chemotherapeutic agent or combination of such agents.

The cause, in contrast to mechanism, of spontaneous variations among individuals of a population is not known. The "reason" variants arise is beyond our comprehension, but the manifestations of their existence are clear in certain instances. Some mutations prohibit the survival of the individual organism;

they may be obviously lethal regardless of the environment, or else so detrimental that the individual cannot survive for long in a particular competitive environment. Other mutations may have little effect on the survival of an organism, but a few mutations have survival value and enable an individual to survive under conditions which are less favorable for survival of the majority of a population. In this last category are the drug-resistant types of bacteria. As Frobisher (33) has stated, bacteria were probably among the first living things and, unless some cataclysmic event eliminates all living things at once, they will probably be among the last to survive. No small part of this ability to survive is caused by the great diversity of variants produced in a relatively short period of time.

We are not qualified to discuss the changes in metabolism which may enable an individual bacterial cell to survive exposure to high concentrations of penicillin or streptomycin. There is, generally speaking, a paucity of knowledge concerning the basic mechanisms involved. The reader is referred to recent articles by Goldstein (34), Linz (35), and Plough and Grimm (36) in which the present concepts and findings are discussed. Attention should also be given to the recent work of Voureka (37), who demonstrated that certain penicillin-resistant bacteria were readily made sensitive to the drug by growing the insensitive cells in mixed culture with other organisms, either penicillin-sensitive or penicillin-resistant. The "sensitization" could be induced in the absence of growth. Also, certain bacterial autolysates produced the change. Winner (38), in similar studies, was able to show quantitatively that an increase in sensitivity to penicillin of a relatively resistant *Staphylococcus* occurred after four hours of contact with a sensitive *Streptococcus*. Most important, the *Streptococcus* became relatively resistant to penicillin after this procedure. Winner suggests that sensitivity to penicillin may depend on some highly labile component of the bacterial cell. Recently George and Pandolai (39) have presented evidence that this labile constituent may be ribonucleic acid. These studies represent a most significant finding which must be correlated with any theory of action of penicillin. Can these findings be a demonstration of another type of bacterial transformation similar to that noted by Griffith (40), Dawson (41), and Avery, MacLeod, and McCarty (42) with the pneumococcus? Attempts in this laboratory to alter resistance of *M. ranae* to streptomycin, using procedures suggested by the studies of Winner and Voureka, have had no success (32). Nevertheless the problem deserves further study. It may be that some simple, but as yet overlooked, procedure may sensitize streptomycin-resistant microorganisms.

THE RESISTANCE OF MYCOBACTERIA TO STREPTOMYCIN IN VITRO

Although the factors affecting the selection of resistant variants from bacterial populations have been studied in detail *in vitro* and the principles are now well established (10, 12, 13, 43, 44), many variables involved in the phenomenon of resistance of mycobacteria to antibacterial agents have not been emphasized sufficiently. An understanding of these variables may prove helpful in the clinical approach to the chemotherapy of tuberculosis.

It has been determined that the detectable number of resistant cells in mycobacterial populations never previously exposed to streptomycin is related to the size of the population and the concentration of the drug. In addition, it has been demonstrated that bacteria must multiply if the number of drug-resistant variants is to increase (45, 46, 47). It should be emphasized that the factors being discussed pertain to *in vitro* studies which can be conducted under carefully controlled experimental conditions.

Recently the writers have demonstrated that, for acid-fast bacteria, the relationship between population size and the number of resistant variants is a direct, linear relationship for any specific concentration of the drug. It is important to emphasize the magnitude of the bacterial populations employed in these studies; the bacteria were numbered in millions and hundreds of millions. Thus, several reported failures to isolate streptomycin-resistant tubercle bacilli from cultures never exposed previously to streptomycin may well have been due to a failure to use sufficiently large populations. In the writers' studies no cultures were found of the type in which the presence of a few streptomycin-resistant bacteria could not be demonstrated, provided the sample sizes were large and many samples were analyzed. Little significance can be attached to the failure to find resistant forms in a single small sample taken from a bacterial population. It is readily shown that when a sufficient number of samples from the same bacterial culture is analyzed, the incidence of streptomycin-resistant forms follows the usual distribution curve characteristic of biological phenomena. This fact gives rise to additional confidence in the belief that the experimental procedures are satisfactory, and offers a ready explanation for the need of analyzing many samples before drawing conclusions as to the presence or absence of streptomycin-resistant bacteria in a culture.

Different cultures of the same strain of mycobacteria analyzed by the same procedure vary considerably in the number of streptomycin-resistant bacteria which can be detected, and these cultures show further variation on subculture. This is similar to the fluctuation phenomenon (2) and is a characteristic to be expected with rare, undirected mutations.

In addition to the size of the bacterial population, the concentration of the drug is important. With low concentrations many streptomycin-resistant variants are found, while with higher concentrations only a few are found. When the concentration of the streptomycin is increased beyond 100 γ per ml. of medium, the number of streptomycin-resistant variants does not decrease appreciably; apparently the majority of tubercle bacilli resistant to this concentration of streptomycin are also resistant to much higher concentrations.

As streptomycin-resistant tubercle bacilli multiply in low concentrations of streptomycin, more forms resistant to higher concentrations of streptomycin appear. This increase in degree of resistance of tubercle bacilli to streptomycin is similar to that of *Staphylococci* to penicillin and streptomycin (9, 10). Demerec noted marked variability in the degree of resistance among first-step streptomycin-resistant strains of *Staphylococci*, and the writers have found that this holds true for mycobacteria. Further studies (30) have demonstrated that when a population of *M. ranae* is exposed to different concentrations of streptomycin

and the percentage of cells which are not able to initiate growth is plotted against the log streptomycin concentration, a sigmoid curve results. In a previous communication (45) it was shown that exposure of tubercle bacilli to streptomycin in relatively low concentration *in vitro* inhibits subsequent multiplication of actively dividing, but not of nondividing, tubercle bacilli when they are transferred to medium free of streptomycin. Subsequent studies have shown that the number of resistant forms in a population of tubercle bacilli exposed to streptomycin does not change when there is no multiplication of the cells. These effects of streptomycin have clinical implications which will be discussed later.

Recent studies in this laboratory using tetrazolium salts have shown that streptomycin is more effective against *M. ranae* when the liquid Tween-albumin culture being tested has a relatively large air-water interface per unit volume. Thus bacteria in 10 ml. of a liquid culture contained in a flask are more readily affected by streptomycin than the bacteria in 10 ml. of the same culture in a test tube of small diameter. This finding is being studied in detail.

These and, undoubtedly, many others are factors which are difficult to evaluate in clinical studies. Nevertheless, there is as yet no reason to believe that a lack of correlation exists in the manner of operation of basic biological mechanisms *in vitro* and *in vivo*.

THE RESISTANCE OF MYCOBACTERIA TO STREPTOMYCIN IN VIVO

It is desirable to indicate in what ways *in vivo* studies are more complex than *in vitro* studies and to show how *in vitro* findings are useful. Does selection of streptomycin-resistant tubercle bacilli occur *in vivo* as it does *in vitro*? The answer would appear to be that it does. The factors, so clearly operative *in vitro*, are more variable under *in vivo* conditions and thus specific results are not readily predicted although the general principles appear to hold true. At present no alternative mechanism has been proposed which operates *in vivo* and not *in vitro*.

With *in vitro* studies the population size is important and can be readily determined, but this is obviously impossible *in vivo*. In certain instances the number of bacteria in the sputum may give a general idea of the size of the bacterial population in the patient, but this method of gross estimation is clearly unreliable. There may be extensive areas of disease containing numerous tubercle bacilli, and only relatively few bacteria from these areas may reach the sputum. Certain patients with cavities in the lungs may provide ideal conditions for the production of immense numbers of tubercle bacilli. During treatment with streptomycin these multiplying populations of tubercle bacilli may be exposed to the drug (48), and the sputum cultures usually become highly resistant (49).

For several reasons it is not possible to determine with accuracy the resistance of the population *in vivo*. Changes are constantly occurring as bacterial reproduction occurs, and the rate of reproduction may not be the same in all parts of the diseased areas. The host is continually influencing the character of the population; the host-parasite relationship results in a reduction of certain bacteria by the host. If extensions of the disease occur, the character of the

population may be altered. Furthermore, tubercle bacilli in some of the diseased areas may be in a resting stage and not be adequately affected by streptomycin. As mentioned previously, *in vitro* studies indicate that streptomycin is not appreciably bactericidal for nonmultiplying tubercle bacilli. If this is true *in vivo*, the sensitive cells may later multiply and produce extensions of the disease. In short, because of the variability of environmental factors *in vivo*, it is impossible to determine the resistance or susceptibility to streptomycin of many of the bacteria in a patient.

Effect of streptomycin concentration: The determination of the concentration of streptomycin in the test tube is simple, but no method is known for determing the concentration of the drug affecting the bacilli in the patient. The drug concentration in body fluids can be determined, but there is no way of determining the association of these fluids with individual bacilli or with groups of bacilli.

With low concentrations of streptomycin, more resistant variants survive than with high, and these produce variants with greater resistance to higher concentrations. This would suggest the desirability of initial treatment with large doses of the drug, but *in vitro* studies (43, 44, 50) indicate that, even when a high concentration is obtained, a few cells may be expected to survive provided the size of the population is large enough. This does not mean that, in theory at least, the body cannot be freed of all tubercle bacilli when streptomycin is used. The host normally destroys tubercle bacilli and as yet there is no proof that the few cells highly resistant to streptomycin cannot be among those destroyed by the host.

It is readily demonstrated *in vitro* that following the first exposure to streptomycin a certain number of bacteria are killed or inhibited. Thus, as soon as streptomycin exerts action on susceptible organisms, selection has begun, and the longer the process continues the more the streptomycin-resistant cells will multiply until finally the population is composed almost entirely of resistant cells. Recently streptomycin therapy has been given for periods as short as two or three weeks, and frequently a significant clinical response is observed. Many of the bacteria are probably adversely affected by the drug during the treatment. It must not be concluded that, because the bacteria in the sputum are said to be still sensitive to streptomycin at the end of two or three weeks of treatment, selection has not occurred. Because selection has begun, it is likely that the future duration of usefulness of streptomycin in these patients has been abbreviated. Intermittent therapy or therapy with very small doses theoretically offers favorable conditions for streptomycin-resistant cells to multiply. Clinical trials, however, must determine the optimum treatment schedules.

In vitro sensitivity tests: The results of the usual "sensitivity test" can be misinterpreted. The test is designed to select the streptomycin-resistant cells, and allows the streptomycin-sensitive cells to go undetected. Also, streptomycin-sensitive cells from disease foci may not reach the sputum. At a later date these sensitive bacteria or their progeny may cause an extension of disease in the treatment of which streptomycin would be effective. This situation and the

natural course of the disease may partially account for some of the conflicting reports on the use of streptomycin in "re-treatment cases." Recently the Veterans Administration (51) reported that cultures of tubercle bacilli obtained from different parts of a resected lung of a patient who had received streptomycin therapy showed marked variation in their sensitivity to streptomycin. Also, it has been observed that tubercle bacilli from the cerebrospinal fluid may be sensitive to streptomycin while tubercle bacilli isolated at the same time from other areas in the same patient may be resistant to the drug (52). The pathologist who obtains specimens of tubercle bacilli from different areas of the lung and other organs in an attempt to find variations in degrees of resistance is confronted with a difficult problem. The technical difficulties in fulfilling the theoretical need for culturing bacteria from numerous extremely small foci are very great.

There are some workers who would like the clinical sensitivity test to be an exact scientific tool, and this has led to various modifications of technique. A detailed discussion of the advantages and disadvantages of using solid medium or liquid medium, direct inoculation of the sputum or subculturing, oleic acid albumin medium or other media, et cetera, is beyond the scope of this article. We shall comment on only a few points which seem to be of fundamental interest.

Recently the writers had the opportunity to review the results of sensitivity studies done in conjunction with a clinical study of streptomycin. The tests were performed by inoculating tubercle bacilli growing in liquid Tween-albumin medium on slants of solid medium containing streptomycin. A considerable number of control tubes (no streptomycin) were recorded as showing only 1 plus or 2 plus growth at fourteen days while the majority showed 4 plus growth at fourteen days. Certain control tubes never reached 4 plus growth after thirty-five days' incubation. There was an occasional report which showed an earlier growth in a higher concentration of streptomycin than in the next lower concentration. Tubes showing less than 4 plus growth were read at intervals until a total of five weeks' incubation occurred. These findings make the tests extremely difficult to control. If approximately equal-sized inocula are used, how can one control tube show 4 plus growth while another shows 1 plus growth? Either the inocula do not contain approximately equal numbers of viable cells or there is a marked variation in the cultural conditions to which the cells are exposed. While some tubercle bacilli may grow more rapidly than others, it is difficult to explain these findings on such a basis alone because when sensitivity tests were done on cultures from one patient at intervals of a few weeks, the control tubes showed growths of 1 plus, 1 plus, 3 plus, 2 plus, 1 plus. It was also observed that growth was occasionally greater in tubes containing streptomycin than in the control tubes. It is not likely that this resulted from the presence of streptomycin-dependent cells because there was no growth or poor growth in certain of the other tubes containing streptomycin which were inoculated from the same culture. These findings are not cited in criticism of any particular technique but rather to show the shortcomings of a clinical test even when carefully performed.

It has been shown that a relatively small number of resistant cells may eventually cause a good growth either in liquid or on solid medium provided the time of incubation is sufficiently long. A procedure to determine a crude estimate of the ratio of resistant to sensitive cells is to read the tests at a fixed time when it is reasonable to expect good growth in the control tubes. If this or some other simple procedure is not adopted, there may be considerable difficulty in comparing results from different laboratories.

It has been shown (52, 53, 54, 55, 56) that the point in time at which loss of sensitivity to streptomycin occurs, as determined by *in vitro* tests, can frequently be correlated with failure to obtain further good clinical response to the drug. In this instance the *in vitro* test results appear to be related to the *in vivo* response. Attempts to convert the clinical "sensitivity test" into a quantitative test, however, are perhaps too ambitious when they try to define within very fine limits when streptomycin therapy can or cannot be expected to benefit a patient. It is appreciated that the physician would like to know the exact sensitivity status of the infecting organisms. In general this is possible only when the ratio of resistant to sensitive cells is very low or very high. Because of the principles outlined in the previous section, it will be difficult to develop a truly quantitative test which is simple enough for mass-testing procedures.

It is difficult to compare different "sensitivity tests." Each presents disadvantages and advantages. Ideally a test should simulate *in vivo* conditions, but how are these to be precisely defined? The recommended procedures have been changed at intervals, even during the course of a single clinical study. It would seem important to understand the basic principles of the test and to realize the limitations of any test no matter how refined. At one time the Gaffky count was extensively employed, and importance was attached to slight variations expressed as Roman numerals. More careful study has revealed the limitations which must be placed on the interpretation of these changes. The clinical "sensitivity test" is now in need of similar evaluation. It should be regarded simply as another useful laboratory aid in the study of the chemotherapy of infectious disease. The value of the test depends more on the appreciation of its limitations than on attempts to convert it to a quantitative procedure.

PERMANENCE OF RESISTANCE TO STREPTOMYCIN, REVERSE MUTATION, AND STREPTOMYCIN DEPENDENCE

Once resistance to a chemotherapeutic agent is established, it persists for some time when cultures are grown in the absence of the drug. This is not unique with streptomycin resistance, but was noted early in the study of chemotherapy and more recently by many workers in studies with sulfonamides and penicillin. Considerable knowledge has been accumulated concerning the action of sulfonamides and the mechanism of resistance to those drugs (34). Much less is known about the mechanisms of the development of resistance to penicillin and streptomycin. Persistent penicillin resistance in certain bacteria is related to the presence of variants producing penicillinase, yet other penicillin-resistant bacteria do not produce penicillinase (57, 58, 59). Recent observations (39) have shown that

resistance to penicillin in certain bacteria appears to be related to the quantity of ribonucleic acid in the cells. The biochemical changes causing streptomycin resistance are not apparent as yet, although some clues have been obtained (60, 61, 62).

The resistance of mycobacteria to streptomycin is usually considered permanent and can be shown to persist after many subcultures are made in the absence of the drug and after passage of the cells through animals (45, 63). This does not mean that streptomycin-sensitive cells do not occur in predominately resistant populations, but at present no adequate laboratory procedure is available for studying reverse mutation quantitatively in streptomycin-resistant tubercle bacilli. This stems from the fact that there is no differential medium which allows growth of susceptible cells while preventing growth of resistant cells. If the rate of reverse mutation were high, a study could be done by a plating method using medium containing streptomycin in some plates and medium containing no streptomycin in others.

Permanence of resistance of mycobacteria may be closely related to the degree of resistance. Certain cultures with a low degree of resistance may be less stable than cultures having a high degree of resistance. This is true of certain nonacid-fast bacteria. It has been observed in this laboratory that cultures of *Serratia marcescens* (# 267) resistant to 10 of streptomycin per ml. of medium are unstable with reference to resistance, while cultures of this same species resistant to 100 γ of the drug per ml. are much more stable and have a low rate of reverse mutation to sensitive cells. It would be of value if studies were made to determine if any similar correlation exists for tubercle bacilli. Several observations have been made on the loss of streptomycin resistance on subculturing tubercle bacilli but no report has yet been made of a study using isolated resistant clones selected at the beginning of the study. Cultures of isolated clones of different degrees of resistance should be subcultured for long periods of time in the absence of the drug and tested and compared systematically. Undoubtedly work of this type is being done by laboratories collecting cultures of resistant tubercle bacilli. In doing an experiment of this type it is important to know that populations of cells resistant to low concentrations of streptomycin contain some cells resistant to much higher concentrations.

Streptomycin dependence: Streptomycin-dependent forms were first isolated from nonacid-fast bacteria (11, 64). The isolation of a streptomycin-dependent strain of *M. ranae* from a streptomycin-resistant strain made it possible to make quantitative studies of reverse mutation to the streptomycin-susceptible type (32). The susceptible variants were readily selected from a culture of the streptomycin-dependent strain because they grew in medium that did not contain streptomycin. Streptomycin-susceptible variants were found consistently in populations of streptomycin-dependent cells, the number varying with the concentration of streptomycin in which the streptomycin-dependent cells were growing. The incidence of streptomycin-resistant cells in cultures grown from these streptomycin-susceptible variants was the same as in the original parent streptomycin-susceptible cultures. This technique, admittedly an indirect ap-

proach, is the only one yet available for the quantitative study of this problem with mycobacteria.

It was found that one streptomycin-dependent strain of *M. ranae* was sensitive to sulfonamides, while another was resistant to sulfonamides. It is well known that genetic changes in one characteristic may be accompanied by changes in other characteristics. The growth rates of the streptomycin-susceptible and streptomycin-resistant variants of *M. ranae* are more rapid than those of the streptomycin-dependent variants. Recently it has been noted in this laboratory that cells in streptomycin-resistant strains vary greatly in their ability to grow on different media. Apparently metabolic variations such as these occur frequently among cells in large populations, and this greatly increases the difficulty of studying the problem of reverse mutation.

Streptomycin-dependent microorganisms have provided another tool to the bacteriologist in the study of bacterial genetics. This can be illustrated by the following observations. *M. ranae* is sensitive to low concentrations of both streptomycin and sulfonamides. It was stated in a preceding paragraph that the streptomycin-dependent variant of this organism will give rise consistently to variants that resemble the parent streptomycin-sensitive strain. The problem becomes more complex, however, if we select a sulfonamide-resistant variant and from this obtain a streptomycin-dependent variant. In this instance we are dealing with two characteristics, resistance to sulfonamides and dependence upon streptomycin for growth. Such a variant presents peculiar colony formation and has a very slow rate of growth. When large populations of this variant are inoculated on the surface of nutrient glycerol agar containing no streptomycin, a relatively large number of isolated colonies appear after a period of twenty days' incubation. Three morphologically distinct colony types are discernible.

In our experiment the streptomycin and sulfonamide sensitivities of many of these colonies were tested. The results were as follows: 75 per cent were resistant to both drugs, 20 per cent were sensitive to streptomycin and resistant to sulfonamides, and the remaining 5 per cent were sensitive to both drugs. The growth characteristics of this last type differed distinctly from those of the original parent type. Thus we have a situation somewhat analogous to segregation in multicellular organisms. The details of this study will be reported later in a separate communication.

The isolation of streptomycin-dependent tubercle bacilli from experimental animals and humans has been reported (65, 66). On the basis of the observation that streptomycin-dependent *M. ranae* grows more slowly than either the resistant or sensitive cells, a simple *in vitro* technique was developed for the isolation of streptomycin-dependent *M. ranae* from a strain never previously exposed to streptomycin. A similar technique was successfully applied to the isolation of streptomycin-dependent tubercle bacilli from strain H37Rv which had not previously been exposed to the drug (67). When large numbers of streptomycin-dependent tubercle bacilli were inoculated on medium containing no streptomycin, a few colonies were consistently found growing in the absence of streptomycin. Cells from these colonies were tested and found to be strepto-

mycin-resistant, in contrast to the reverse mutants obtained from streptomycin-dependent *M. ranae* which were streptomycin-sensitive. The clinical and biological significance of streptomycin-dependent tubercle bacilli may now be more readily determined with the use of this technique.

It was found that a population of streptomycin-dependent bacteria obtained from a single clone was not homogeneous with respect to the streptomycin requirements of the individual cells. The majority of the cells grew in the original concentration of the drug from which the dependent variant was selected, and a small number of the cells were capable of growing in higher concentrations of the drug. In addition, a few cells were found which would grow in the presence of a minute amount of streptomycin and a very few (one to 8 per 100 million) which would grow in the absence of streptomycin. These latter tubercle bacilli were uniformly resistant to streptomycin. Thus, by inoculating large numbers of streptomycin-dependent microorganisms in medium without streptomycin, it is possible to obtain quantitative data on the occurrence of reverse mutation.

As stated previously, it is not yet possible to determine quantitatively reverse mutation from a streptomycin-resistant variant. From a biological as well as a clinical viewpoint it would be of much interest if conditions could be provided under which only the streptomycin-sensitive cells could be grown from the streptomycin-resistant population. Since there seems to be no difference in colony morphology between streptomycin-resistant and streptomycin-sensitive strains of tubercle bacilli, it was decided to work with a pigment-forming strain of bacteria which produced different color types when inoculation from a single colony was made on solid medium (21). When a streptomycin-resistant colony was selected from a particular color variant and plated on medium without streptomycin, any colony having a color different from that of the original streptomycin-resistant type could be tested for sensitivity to the drug. *S. marcescens* (American Type Culture Collection Strain #274) was selected for the study.

When this microorganism is plated on a synthetic solid medium, four distinct colony color variants appear: dark red (DR), bright pink (BP), pale pink (PP), and slightly pigmented white. Only rarely can a stable white colony be isolated. The progeny of a DR colony is comprised of about 95 per cent DR and 5 per cent BP. A BP colony will produce definite proportions of BP, DR, and PP, and similarly a PP colony will produce definite proportions of the other color variants. The reader is referred to the papers of Bunting (19, 20, 21) for detailed studies on the color variants of *S. marcescens*.

Streptomycin-resistant colonies of each color variant were selected by the pour plate method from medium containing 100 γ of streptomycin per ml. It was found by appropriate experiments that a single resistant colony was not homogeneous with respect to the colony color of its progeny; however all the color variants obtained were resistant to streptomycin. The proportion of color variants obtained from a single resistant colony was, in most instances, markedly different from that of the original streptomycin-sensitive parent strain. It was concluded that the determinant mechanisms for color and for streptomycin resistance are separate and that resistance is transmitted regardless of color type.

It was stated previously that the occurrence of a stable white colony is rare indeed and is never seen in populations derived from a DR colony. During the process of isolating streptomycin-resistant colonies from the progeny of DR colonies, the incubation of the plates containing streptomycin was prolonged and many white colonies appeared. These were found to be of two types; one resistant to, and the other dependent on, streptomycin.

The streptomycin-dependent colonies uniformly produced white colonies even after many subcultures in the presence of streptomycin. Each streptomycin-dependent white colony contained a few cells capable of growth in the absence of streptomycin, however, and these cells gave rise to all four color types: DR, BP, PP, and white.

It is interesting that even apparently stable white colonies contain a few cells capable of forming the various pigments when the proper environmental conditions are provided. Perhaps the origin of these white colonies from the DR streptomycin-resistant variant is of even greater significance. As mentioned previously, no white colonies derived from the streptomycin-sensitive DR type were seen even after prolonged incubation. It is believed that the white, streptomycin-dependent colonies are derived from mutants which occur in the parent population but do not grow in the absence of streptomycin.

It is evident from these observations that forward and reverse mutations can occur. Because of the lack of an experimental procedure, it can only be assumed on the basis of general biological phenomena that reverse mutation may also occur from streptomycin-resistant variants.

It has been noted in this and in other laboratories that cultures from a few patients may be resistant to streptomycin at the end of a course of streptomycin therapy, but several months later new cultures from these patients are found to consist predominantly of streptomycin-sensitive cells. These findings pose an important problem which deserves careful study. It is not sufficient to say that the cells have "changed" or to make the simple statement that reversion has occurred. The sensitive cells may be from diseased areas which were either inaccessible to effective concentrations of streptomycin or contained resting cells which remained sensitive to streptomycin. Also, if individual colonies are picked from solid medium for sensitivity tests, the possibility of picking an occasional streptomycin-susceptible colony should not be overlooked. And finally, true reverse mutation may have occurred.

It has been shown that the *in vitro* growth rate of at least one strain of streptomycin-resistant tubercle bacilli is slower than that of sensitive cells derived from the same parent strain (68). If reverse mutation is occurring, a similar differential in growth rates *in vivo* would be highly significant, and in some instances the sensitive cells might become the predominant type in the population. The growth rate differences may be influenced greatly by the exact location of the bacteria in the diseased areas with specialized environmental conditions.

MULTIPLE DRUG THERAPY

Variants are present in every biological population. Regardless of the reason for their occurrence, the ultimate effect appears to benefit the population in terms of survival in its environment. Certain bacterial variants, and in particular tubercle bacilli resistant to streptomycin, are distinctly not of benefit to man, no matter what benefit they may be to perpetuation of the bacterial species. It is generally agreed (69) that 50 to 70 per cent of patients who continue to have tubercle bacilli in their sputum after 120 days of treatment with streptomycin will have streptomycin-resistant strains. In addition it has been shown both in experimental animals and clinically that streptomycin is generally ineffective against

infections with these resistant strains (52, 53, 54, 63, 70, 71). Obviously, any procedure such as combined drug therapy which would diminish or avoid the development of streptomycin-resistant tubercle bacilli will be exceedingly valuable. Among the earliest reports of the effective action of two or more drugs used simultaneously against mycobacteria are those of Smith and McClosky (72) who used streptomycin and Promin in experimental tuberculosis in guinea pigs and of Middlebrook and Yegian (45) who studied the combined action of streptomycin and sulfathiazole against *M. ranae* and of streptomycin and diaminodiphenylsulfone against the tubercle bacillus.

The population size required to produce a variant resistant to two or more drugs used concurrently may be immense when each drug used individually is effective against the microorganism. *M. ranae* is susceptible to streptomycin and to sulfonamides. When a streptomycin-resistant strain was studied, it was found that the incidence of sulfonamide-resistant bacteria was the same as in the parent strain which had never been exposed previously to either drug. Similar findings resulted when sulfonamide-resistant cells were studied for resistance to streptomycin. When cultures of *M. ranae* were exposed to a combination of 1.0 γ of streptomycin per ml. of medium and 1.0 mg. of sulfonamide per 100 ml. of medium, it was not possible to select variants resistant to the combination of the two drugs, whereas it was a simple matter to select variants resistant to either drug when used alone. Theoretically it is possible to obtain a variant resistant to the two drugs when both are present in the medium, but in practice it may be difficult. Only by exposing a culture of *M. ranae* to a very low concentration of both drugs and making successive subcultures of the surviving organisms in gradually increasing concentrations was it possible to obtain variants resistant to high concentrations of both drugs.

At the present time considerable laboratory and clinical work is in progress to study the resistance of tubercle bacilli to various combinations of drugs. The principles of combined drug therapy, of course, are not new but were proposed and studied carefully by Erhlich (18). Combined drug therapy has been employed in many infectious diseases, but until recently it has not been applied to tuberculosis, principally because of the paucity of drugs clinically useful against the tubercle bacillus. With the advent of para-aminosalicylic acid (PAS) as a useful chemotherapeutic agent, effective combined drug therapy in tuberculosis may become a reality. Other agents, such as neomycin and sulfones, may well prove to have maximum clinical usefulness when combined with streptomycin or other drugs.

Para-aminosalicylic acid: Bernheim's observation that the oxygen uptake of tubercle bacilli was increased by sodium salicylate and sodium benzoate (73, 74, 75) led Lehmann to examine the action of closely related compounds with the hope of finding a substance effective against the tubercle bacillus. Of more than 50 chemicals studied, the most active bacteriostatic compound was para-aminosalicylic acid (76, 77, 78, 79). Lehmann found that PAS was active *in vitro* in experimental tuberculosis and in the treatment of clinical disease. His observations have been confirmed both in Europe, where most of the early work with the drug was done, and in this country (80, 81, 82, 83, 84, 85).

It has been the general experience thus far that only mammalian types of tubercle bacilli are highly susceptible to the antibacterial action of PAS, and among these there is rather wide variation in the degree of susceptibility. It has been reported that PAS is bacteriostatic at relatively low concentrations against streptomycin-resistant as well as streptomycin-sensitive tubercle bacilli (81, 86). Studies of the combined action of streptomycin and PAS against tubercle bacilli both *in vitro* and *in vivo* have been interpreted by some workers as demonstrating an additive effect and interpreted by others as indicating a synergistic effect (86, 87, 88). Recently it has been stated (89) that the addition of PAS to streptomycin prevents or greatly retards the *in vitro* development of streptomycin resistance and that "repeated exposure of *M. tuberculosis* to PAS for 120 days failed to produce an increase in the resistance of this strain" to PAS.

In this laboratory certain of the above observations have been confirmed and some new information gained. In one experiment 12 series of plates were used; each series contained 16 plates. In each of 4 series a different concentration of streptomycin was used (0.5, 1.0, 10.0, 100 γ per ml. of medium); in 4 other series PAS was used (0.5, 1.0 10, 100 γ per ml. of medium); in 4 other series PAS and streptomycin were used in the same medium (0.1 γ streptomycin plus 0.1 γ PAS per ml. of medium; 0.25 γ streptomycin plus 0.25 γ PAS per ml. of medium; 0.5 γ streptomycin plus 0.5 γ of PAS per ml. of medium; 1.0 γ streptomycin plus 1.0 γ PAS per ml. of medium). Each plate in each series was inoculated with approximately 200 million tubercle bacilli strain H37Rv and incubated at 37°C.

The usual number and distribution of colonies growing in the presence of the different concentrations of streptomycin were noted at 35 days' incubation. An average of 220 colonies was growing on each plate containing 0.5 γ of streptomycin per ml., and an average of 15 colonies per plate on the series containing 1.0 γ of drug per ml. A few colonies were noted growing on certain of the plates containing 10 and 100 γ of streptomycin per ml. These findings were in contrast to those noted with PAS. There was a total of 8 colonies growing on the series of plates containing 0.5 γ of PAS per ml. of medium and a total of 4 colonies growing on the series containing 1.0 γ of the drug per ml. No colonies were found growing on plates containing higher concentrations of PAS at 35 days' incubation. Each of the colonies growing on the plates containing PAS was tested in liquid medium containing PAS and was found to produce good growth in the presence of 1.0 γ of the drug per ml. On the plates containing 0.1 γ of both PAS and streptomycin per ml. of medium, a confluent growth was obtained, whereas only 2 colonies were noted in the entire series of plates containing 0.25 γ of each drug per ml. of medium. No colonies were observed in the two series containing higher concentrations of the two drugs.

These studies indicate that a few cells in large populations are resistant on the first exposure to a concentration of PAS which is inhibitory for the vast majority of the cells. When the plates containing low concentrations of PAS were kept for 45 or more days, it was observed that a confluent growth began to appear. These cells were *not* resistant to PAS when tested in liquid medium. The significance of this is being studied. It should be mentioned that when PAS is used there is a small "initial growth" which must not be mistaken for the growth of resistant cells. This phenomenon is analogous to the "lag-phase" of bacteriostasis of many organisms by sulfonamides.

The clinical use of combined therapy in tuberculosis with PAS and strepto-

mycin (and in some cases, Promin) was reported recently by Karlson *et al.* (68). After treating 14 patients for three months with either PAS and streptomycin or PAS, streptomycin, and Promin, 12 patients had tubercle bacilli in the sputum and of these only one had organisms resistant to more than 1.0 γ of streptomycin per ml. of medium. After six months treatment only 3 additional patients had tubercle bacilli in the sputum which were resistant to streptomycin. Although the number of patients in the series is very small, the streptomycin resistance findings are in contrast to those obtained when streptomycin

TABLE 1

Comparison of the Activities of Neomycin and Streptomycin on Mycobacterium Tuberculosis Strain H37Rv

NUMBER OF BACTERIA PER INOCULUM IN MILLIONS	DISTRIBUTION OF COLONIES AMONG PLATES	TOTAL NUMBER OF RESISTANT COLONIES	AVERAGE PER PLATE
	Number of colonies growing on each plate containing 5 units of neomycin* per ml. of Tween-albumin medium		
290	2 5 5 5 4 1 3 3 3 2 5 3 5 3 0 8 3 4 4 3 2 4	77	3.5
	Number of colonies growing on each plate containing 5 units of neomycin per ml. of oleic acid albumin medium		
290	104 123 131 101 85 115 103 95 75 105 109 115 123 137 107 98 102 117 131 112 117 109	2,414	110
	Number of colonies growing on each plate containing 5 γ of streptomycin per ml. of Tween-albumin medium		
290	7 4 4 3 4 1 5 4 2 2 5 5 2 3 7 3 6 4 4 3 4 2	84	3.8
	Number of colonies growing on each plate containing 5 γ of streptomycin per ml. of oleic acid albumin medium		
290	4 2 4 1 2 2 4 1 4 2 3 5 5 2 5 0 4 5 3 3 2 2	65	3.0

* Neomycin Lot 14A (100 units per mg.). The quantity of neomycin was adjusted according to Dr. Waksman's directions so that one unit was equal to 1.0 γ of streptomycin.

is used alone. As yet no other published report has been made on this phase of work, but numerous studies are in progress to evaluate fully the effect of combined therapy on the development of streptomycin resistance.

Neomycin: An antibacterial agent effective against streptomycin-resistant as well as streptomycin-sensitive tubercle bacilli and for which there is a very low incidence of resistant cells would obviously be of great value. With such an agent in mind as an ideal, Waksman continued his search for additional antibiotics and recently announced the isolation of neomycin (90). Preliminary experiments indicated that neomycin was highly active against mycobacteria including streptomycin-resistant strains of *Mycobacterium avium* and tubercle

bacillus H37Rv (91). The drug was shown to have a bactericidal action at fairly low concentrations against *E. coli* and to be equally effective against streptomycin-resistant and streptomycin-sensitive cells of this microorganism. With the particular technique employed, no neomycin-resistant forms were detected in a large population of *E. coli*.

The writers have had the opportunity of performing some preliminary experiments with neomycin[3] to compare the incidence of variants resistant to neomycin and to streptomycin in populations of tubercle bacilli strain H37Rv.

TABLE 2

Comparison of the Activities of Neomycin and Streptomycin on Mycobacterium Tuberculosis Strain H37Rv

NUMBER OF BACTERIA PER INOCULUM IN MILLIONS	DISTRIBUTION OF COLONIES AMONG PLATES	TOTAL NUMBER OF RESISTANT COLONIES	AVERAGE PER PLATE
	Number of colonies growing on each plate containing 10 units of neomycin* per ml. of Tween-albumin medium		
260	4 3 4 1 1 5 6 2 4 3 3 3 7 6 5 5 7 4 4 3 4 2	86	3.9
	Number of colonies growing on each plate containing 10 units of neomycin per ml. of oleic acid albumin medium		
260	3 7 5 7 2 6 4 2 4 4 6 4 3 5 7 5 5 4 4 3 3 2	95	4.3
	Number of colonies growing on each plate containing 10 γ of streptomycin per ml. of Tween-albumin medium		
260	5 4 1 8 2 6 6 6 4 5 3 4 5 1 4 5 4 1 2 4 4 4	88	4.0
	Number of colonies growing on each plate containing 10 γ of streptomycin per ml. of oleic acid albumin medium		
260	4 2 3 2 1 6 5 6 2 5 5 2 1 3 4 4 3 3 4 5 2 4	77	3.5

* Neomycin Lot 14A (100 units per mg.).

Initial experiments were performed using neomycin incorporated in solid oleic acid albumin medium. It was observed that there was a markedly higher incidence of variants resistant to a specific concentration of neomycin than to an equivalent concentration of streptomycin. These findings were confirmed using a second lot of neomycin. Because these results were not comparable with those obtained in his laboratory, Dr. Waksman suggested an experiment to compare the action of neomycin in Tween-albumin medium and oleic acid medium. The results of such an experiment are shown in tables 1 and 2. Table 1 shows clearly that neomycin is much less effective in oleic acid albumin medium than

[3] Neomycin was supplied through the courtesy of Dr. S. A. Waksman.

in Tween-albumin medium. When Tween-albumin medium is used, the number of streptomycin-resistant variants and neomycin-resistant variants are quite comparable. It can be seen in table 2 that the number of variants resistant to either drug in a concentration of 10 units per ml. is essentially the same when either media is used. Apparently after inactivation of a certain amount of neomycin by the presence of oleic acid, there still remained a sufficient amount of neomycin to produce a marked effect. This point remains in need of further study. Also, in table 2 it is shown that there are approximately the same number of variants resistant to 10 γ of streptomycin as to 10 units of neomycin among cells of tubercle bacilli strain H37Rv. It should be mentioned that different subcultures of cells were used in obtaining the results recorded in tables 1 and 2.

In our experience neomycin has been found to be at least as effective against streptomycin-resistant as against streptomycin-sensitive tubercle bacilli. Neomycin-resistant tubercle bacilli were found to be sensitive to streptomycin, PAS, and sulfathiazole.

Choice of regimen of combined therapy: Because the use of combined drug therapy in tuberculosis is relatively new, the most effective schedules for treatment are not yet known. Clinical trials of different regimens will necessarily furnish the final criteria as to which schedules produce maximum therapeutic effect and the longest delay in the emergence of drug-resistant strains. Combined drug therapy may be used from the onset of drug treatment, or one drug may be given for a period of time and then another one (or more) started when the first drug has presumably had maximum effect in terms of reducing the bacterial population size. If the second drug is withheld too long, the bacterial population may increase and consist chiefly of cells resistant to the drug given initially, and resistance to the second drug may occur as rapidly as if it has been used alone; thus, the effectiveness of combined therapy in reducing the incidence of resistant strains will be lost. When streptomycin and PAS are used, it may be advantageous to give streptomycin alone for a period of two weeks, then give both drugs for two weeks, and finally stop streptomycin after a total of four weeks but continue PAS. This schedule has certain theoretical advantages. It is known that tubercle bacilli develop PAS-resistant strains rather slowly and streptomycin might eliminate most of the rare PAS-resistant variants in a short period of time. In addition, streptomycin is bactericidal in certain concentrations for tubercle bacilli which are multiplying (45). It remains to be determined whether the bactericidal action of streptomycin is as great in the presence of PAS which itself is bacteriostatic.

In a population of tubercle bacilli, 4 types of cells may be present with respect to streptomycin and PAS resistance: one type resistant to both drugs, another resistant to neither drug, a third resistant to PAS, and a fourth resistant to streptomycin. Until more clinical experience is gained, sensitivity tests might well include not only streptomycin sensitivity, but also sensitivity to the other drug (or drugs) and to the combination of drugs. If sensitivity to individual drugs only is tested, one may be misled concerning the *in vitro* action of the combination of drugs. In this laboratory a strain of *M. ranae* was found to be resistant

to streptomycin and to sulfonamides when the drugs were used individually in the usual sensitivity tests. When the drugs were used in combination, growth was inhibited. Only when cells resistant to the combination of drugs were present was the combination ineffective.

It may be that the development of strains of tubercle bacilli resistant to the combination of PAS and streptomycin will not readily occur clinically; at least that is the present hope. We have shown that PAS resistance can develop *in vitro* in streptomycin-resistant as well as in streptomycin-sensitive strains. Recently it has been shown (92) that PAS resistance occurs when patients having streptomycin-resistant tubercle bacilli are treated with PAS for long periods of time. As yet no reports have cited the development of strains resistant to the combination of drugs when both drugs are given in combination to a patient who has never previously had either drug. At this point it might be well to emphasize again that the test tube is an artificial environmental situation and the *in vitro* results must not be overemphasized.

The investigation of combined drug therapy in tuberculosis has only begun and many interesting possibilities remain. Not the least of these is the combining of several agents chemically so that the active components are all available in a single substance. The late Doctor Winnek succeeded in combining streptomycin and Promin and was attempting to combine streptomycin and other antibacterial agents in such a manner that the active components of the individual drugs would be available. Whether this will be feasible and result in highly effective agents is not known, but the clinical usefulness of a single drug to replace two or three is readily apparent.

GENERAL DISCUSSION

Geneticists generally consider that there are three modes of genetic variation: recombinations, aberrations, and mutation. It is not known by which of these processes streptomycin-resistant variants are produced, but there is much evidence that it is by mutation. Evidence at the present time points to hereditary change rather than adaptation (environmental modification) as the mechanism of the production of resistant variants. Nevertheless, although most bacterial variations appear to occur spontaneously rather than as a response to the environment, the latter influence plays an important role in determining the course of bacterial variation (2). The incidence of a specific variation in a wild-type bacterial population is so low as to be relatively unimportant from a clinical point of view until environmental influences are exerted to select these variants. The influence of environment has been studied carefully *in vitro*, but much remains to be learned. Certainly it is obvious that the environment of bacteria within a host is much more complex and difficult for specific analysis than in a test tube.

Streptomycin-resistant tubercle bacilli are variants of the general bacterial population of tubercle bacilli. In addition to the obvious difference between streptomycin-resistant cells and parent-susceptible cells, there are other differences between these cells. Some of these known, or as yet unknown, differences

may be important in the host-parasite relationship. These differences may conceivably bring about changes in the evolution of the disease. Investigators have noted no appreciable changes in the virulence of these cells, but as yet the methods for studying the disease in the laboratory are inadequate to draw conclusions as to the long-term effects on the nature of the disease in humans.

At the present time many of the investigations in tuberculosis research are concentrating on chemotherapy. This field has produced marked advances in therapy, but the development of drug-resistant tubercle bacilli has re-emphasized the importance of a broader approach to the treatment of the disease. Until a drug is found—and there is no guarantee that one will be found—which will eliminate all tubercle bacilli from the infected patient in a relatively short period of time, it would seem reasonable to devote more time to immunological studies in an effort to eliminate drug-resistant tubercle bacilli by host-defense mechanisms.

Streptomycin-resistant tubercle bacilli are rapidly becoming the predominant type in certain patient populations and in time they may replace the original parent-type cells. Are there any procedures to enhance the immunological mechanisms of patients sufficiently so that they eliminate the drug-resistant cells at the time when they are fewest in number, i.e., early in the course of drug therapy? Intensive efforts should be applied to this problem. As yet no one has adequately studied the antigenic properties of drug-resistant and drug-dependent tubercle bacilli. Also, the subject of reversion of drug-resistant cells to drug-sensitive cells deserves attention. There may exist a substance which can sensitize streptomycin-resistant tubercle bacilli in a manner analagous to the sensitization of penicillin-resistant organisms. The fact that the specific techniques used in the penicillin studies failed with mycobacteria and streptomycin should not be discouraging. Some simple technique, as yet undiscovered, may solve the problem.

It may well be anticipated that new cases of tuberculosis infected with streptomycin-resistant bacteria will occur. Recently two reports have been made of patients who appear to have developed their disease as a result of infection with highly resistant tubercle bacilli (93, 94). The writers have been informed that other similar cases have been observed and will be reported. It is a policy to test routinely the streptomycin sensitivity of the tubercle bacilli from all patients admitted to New York State sanatoriums with tuberculosis which has presumably been acquired since 1947. This is similar to a policy already adopted by certain other institutions. Valuable information concerning the epidemiology of the disease may be gained if this study is made *now* before the number of patients infected with streptomycin-resistant organisms constitutes the majority. Knowledge of the streptomycin sensitivity of the tubercle bacilli in new cases is of importance with regard, not only to subsequent treatment, but also to the source of the infection. There is still not unanimous agreement as to the source of tubercle bacilli causing so-called reinfection tuberculosis. It is possible that evidence in favor of exogenous sources may be derived as a result of streptomycin sensitivity studies. The finding of predominately highly resistant tubercle bacilli

in a patient who had had previously arrested disease caused by streptomycin-sensitive organisms would indicate that the reinfection was not of endogenous origin. Perhaps there will be only a few cases studied adequately, but these could give much information as yet difficult to obtain by other means.

Not the least important of the fields of investigation which should be pursued more thoroughly is the study of bacterial variation. Bacterial evolution is, by definition, a constantly changing process. As environmental changes occur, selection values change. This is well demonstrated by the studies of *S. marcescens* described previously in which it was noted that colonies of four different colors occurred in one environment while in another environment (containing streptomycin) colonies of only one color type grew. A number of problems concerning the relationship of various strains of mycobacteria to each other remain. For example, speculation exists concerning the relationship between bovine, avian, and human types of tubercle bacilli, and some have theorized that the various types were originally derived from a common cell. At present many variants of mycobacteria possessing unique characteristics have not been classified. They may exhibit marked differences in virulence (from their parent cells) which have accompanied the morphological and biochemical changes. A strain of nonpathogenic sulfonamide-resistant *M. ranae* exhibits cord formation similar to that of pathogenic tubercle bacilli (95). As anticipated, current experiments are demonstrating striking differences between the virulence of streptomycin-dependent tubercle bacilli and the parent streptomycin-sensitive cells. It is apparent that many newly isolated variants must be tested and evaluated further.

The resistance of tubercle bacilli to drugs is of such fundamental importance that every aid to the study of the problem should be employed. The cooperation and help of investigators working in a variety of the allied fields of science are needed to give the investigation a broad outlook and a fundamentally sound basis. There is little evidence that many years of working with tubercle bacilli should be a prerequisitie to investigation in the field. Indeed the opposite may be true.

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EXPERIMENTAL STUDIES ON THE PATHOGENESIS AND PROGNOSIS OF RENAL TUBERCULOSIS[1]

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INTRODUCTION AND REVIEW OF LITERATURE

The management of genitourinary tuberculosis is frequently unsuccessful if treatment is limited to local lesion. The reason for this lies in the pathogenesis of the disease.

Renal tuberculosis is always part of a generalized systemic disease. The primary infection is extra-renal, i.e., in the lungs, intestinal tract, or elsewhere, and is often completely healed by the time tuberculous involvement of the genitourinary tract becomes apparent. The genitourinary tract itself is a not infrequent source of subsequent hematogenous disseminations. Renal tuberculosis must be treated as one aspect of a systemic disease. The management of the patient will therefore depend on the number of organs involved, the virulence of the infection, the hypersensitivity of the patient, and his resistance to tubercle bacilli, to mention a few factors.

Three forms of renal tuberculosis occur. The first of these consists of involvement with miliary tubercles. Large numbers of these foci are present in both the cortex and medulla of the kidneys in all cases of generalized miliary tuberculosis. Similar foci are present in cases of chronic pulmonary tuberculosis but are less numerous in this form of the disease. Another form is the large nodular type of tuberculous lesion.

These foci vary from one millimeter to one centimeter in diameter and usually show no tendency to liquefaction. The third form is that of an isolated organ tuberculosis as seen in the so-called surgical kidney. It is characterized in the early stages by an ulceration in the region of the papillae.

Tuberculous infection of the kidney is hematogenous in almost all instances. Since infection is hematogenous, involvement may be expected to be bilateral and this has been shown to be the fact by a number of investigators. Medlar and Sasano (1) found renal tuberculosis to be bilateral in 88 per cent of their cases, while in 252 cases analyzed by Greenberger and Greenberger (2), bilateral involvement was present in 82 per cent. In an analysis (3) of one thousand autopsies performed at the Sea View Hospital renal tuberculosis was bilateral in 88.2 per cent of cases in which it occurred. It should be noted that hematogenous infection does not necessarily progress to so-called organ tuberculosis of the kidney. It should be stressed, however, that these figures refer to all three forms of hematogenous seedings.

The nature of the hematogenous infection of the kidney has been studied. Tubercle bacilli were injected intravenously in different nonsensitized animals

[1] From the Urological Service of Sea View Hospital, Staten Island, N. Y.

to determine whether there was a predilection for seeding into certain organs. It was noted that diseased or traumatized areas develop tuberculosis earlier than nontraumatized tissues and it was thought that this was due to *locus minoris resistentiae*. It was found difficult to produce isolated tuberculosis of any organ. Regardless of where the injection of the tubercle bacilli was made, the disease spread through the blood stream and the inoculated organ often showed moderate involvement, while the disease became widespread throughout the body. The role of trauma, however, is not necessarily an important one in the spread of the disease. In this hospital we have observed 11 patients with pulmonary or osseous tuberculosis who had traumatic kidney injury with at least prolonged microscopic hematuria and none of these 11 cases developed kidney tuberculosis.

The original involvement of the kidney is manifested by a small tubercle, which is localized usually in the cortical or corticomedullary area of the kidney. At this time there is no clinical evidence that the kidneys are involved and only the systemic manifestations of the disease are evident. Urine examination is usually negative for tubercle bacilli. This small tubercle may increase in size; caseation may occur and progress through the medulla. When the process reaches the renal papilla, clinical manifestations of the disease appear. The urine contains tubercle bacilli, although urinary symptoms may still not be present. Roentgenographic studies at this stage show no definite pathologic changes.

Tubercle bacilluria, without evident kidney disease, has been widely discussed in previous decades, and has not been universally accepted as an indication of renal tuberculosis. Some authors thought that, in certain cases, where the permeability of the glomerular filter is increased by a nontuberculous infection, tubercle bacilli might pass through an intact glomerular membrane. Thus, two schools of thought existed. One held that tubercle signified a renal lesion, while the other proposed that bacilluria might be the result of excretion of tubercle bacilli through an intact renal filter (4, 5).

It was first proved conclusively by Richards (1917) that filtration of certain substances takes place through glomerulus. The proponents of the "excretory" theory, i.e., the passage of tubercle bacilli through an intact kidney, claimed that these data supported their point of view and noted, in addition, that Andrews and Thomas demonstrated by precipitin reaction that proteins, other than blood proteins, might be present in urine without kidney damage being evident histologically. Rusznyak and Nemeth supplied further experimental evidence to support this theory. They perfused dog kidneys with serum from hypertensive or nephrotic patients and found no albuminuria. However, when saponin or digitalin was added to the perfusing fluid, albuminuria resulted. This they believed to be definite proof that proteins may pass through the glomerular filter. It was noted that ingestion of raw egg might result in proteinuria.

These experimental findings and clinical observations seemingly indicated that under certain conditions tubercle bacilli might pass through an intact glomerulus. The excretory theory, however, was not unanimously accepted. It was many years later that Medlar and Sasano (1) conclusively proved that bacilluria with *M. tuberculosis* indicated kidney tuberculosis. They produced renal tuberculosis in rabbits and injected urine from the

infected kidneys into guinea pigs. They found that tubercle bacilli in the urine always came from a tuberculous lesion in the kidney. Helmholz and Field (8) (1925) recovered bacteria from the urine only after sufficient time had elapsed to produce renal damage, and the renal injury was demonstrated in all cases. Lepper (9) used coliform bacilli and found that intravenous injection of the bacilli caused bacilluria only in those cases in which definite kidney injury could be demonstrated. Lieberthal and Huth (10) in 1933 reported a series of one thousand nephrectomies for tuberculosis and in each case of bacilluria with *M. tuberculosis*, they could demonstrate a definite tuberculous focus, although sometimes serial sections had to be done to demonstrate the lesion.

The usual course of renal tuberculosis, where bacilluria is present and there is roentgenographic evidence of destruction, is a gradually progressive one. Healing, however, can take place, especially in those cases where there is a corticomedullary miliary involvement or where bacilluria is not accompanied by roentgenographic evidence of the disease. Medlar (11) and Auerbach (12) proved that the healing may be so complete that only a microscopic zone of fibrosis will indicate the presence of a previously diseased area. Healing can even occur in those cases where the disease is not localized in the cortex but where minute ulceration of the papilla is present. Beach and Schultz (13) reported 8 cases in which bacilluria disappeared and the patients remained symptom free from six to fifteen years. Sporer (3) studied 17 cases of bacilluria where roentgenographic studies showed no renal pathology and clinical examination failed to reveal genital tuberculosis. In each of these 17 cases the bacilluria disappeared and the patients remained symptom free for more than two years.

Auerbach has shown that the majority of cases of renal tuberculosis are unilateral. As noted above, however, there is no way to diagnose the presence of a small, active cortical lesion on the other side. There is no bacilluria from this side and the roentgenograms reveal no involvement. This lesion may break down and can produce organ tuberculosis at any time. Since the possibility of bilateral disease is always present, how will this influence the therapy? What should be done in the presence of apparently bilateral involvement? What is the prognosis in each of these cases?

Clinical and experimental studies are being pursued at Sea View Hospital for the elucidation of the problem of bilateral renal tuberculosis. Up to the present, prognosis remains uncertain, depending upon a number of factors, especially the type of therapy which is employed.

At the beginning of the twentieth century, several cases were reported in which nephrectomy in bilateral disease not only saved and prolonged the life of the patients but was said to have increased the healing tendency in the remaining kidney. The improvement was explained by the so-called nephrotoxin theory. The observation that albuminuria, cylindruria, and pyuria might disappear postoperatively from the remaining kidney was said to indicate that the diseased kidney produced toxins which in turn damaged the contralateral kidney. If the diseased kidney were removed in time, the lesion in the remaining kidney might not be far advanced and the kidney might return to normal. The general improvement of the kidney thus, it was claimed, enabled the organ to resist the progression of the tuberculous process. Statements were even made by Rochet and Thevenot (14) that albuminuria from the opposite kidney not only does not contraindicate surgery, but is an indication for the operation.

Experimental support for the above claims were found in Uffredazzi's experiments published in 1918 (15). This worker attempted to determine what effect nephrectomy had on bilateral renal tuberculosis. He infected rabbits with tubercle bacilli and produced bilateral renal tuberculosis. In a number of cases, after the infection was established, he performed a nephrectomy. The nephrectomies were done at various intervals after the infection. Some of the animals with bilateral kidney involvement were kept as controls. He stated that animals with bilateral renal tuberculosis in which one kidney was removed had a longer survival period than the control animals. He found that when nephrectomies were performed at an early stage, almost complete healing occurred on the opposite side. The later the nephrectomy, the less was the evidence of healing on the opposite side. If the post-infection period was long, the nephrectomy did not help the opposite kidney. In those cases where no nephrectomy was performed, the disease was a constantly progressive one, with ultimate destruction on both sides. These experiments are widely quoted and have served as the basis of the rather widespread belief that nephrectomy may help the opposite kidney in bilateral renal tuberculosis.

Uffredazzi's work was supported by several later experiments. It was claimed that the damaged renal tissue of one kidney acts as an antigen against which antibodies are formed. These antibodies, unable to distinguish the intact tissue of contralateral organ from the diseased tissue, damage the former and thus, as in *locus minoris resistentiae*, tuberculosis may develop there. Several workers claimed the production of organ-specific antibodies. Forssner (16) cultivated bacteria *in vitro* with tissues of different organs, and, upon injection, claimed that there was a predilection for these bacteria to invade organs similar to those upon which they were grown.

On the basis of these theoretical concepts, numerous cases of bilateral renal tuberculosis have been treated by nephrectomy in the past several decades. Unfortunately no follow-up studies have been reported on the results of these operations. On the service at Sea View Hospital several nephrectomies were performed in patients with bilateral kidney tuberculosis, but the results have not been encouraging. It was not possible to obtain evidence that nephrectomy in these patients helped the condition of the remaining kidney. This seemed at variance with the previously quoted studies and prompted a re-study of the theoretical basis for this idea.

OBSERVATIONS

Experimental Studies in Rabbits

Inasmuch as Uffredazzi's work is fundamental to the concept of removal of the badly diseased kidney, it was decided to repeat his experiments. Uffredazzi injected one drop of a suspension of the bacilli (one loopful of a culture of a ten- to fifteen-day growth on potato medium diluted in one centimeter of physiologic saline solution). In his experiments, this material was injected under the capsule of the kidney intracortically, and produced a pinhead-sized tuberculous nodule approximately forty days after injection. The lesion grew rapidly in the course of ten to twelve months. The extent of the caseation in the focus apparently depended upon the virulence of the bacteria. In the present experiments an attempt was made to eliminate all source of experimental error. Cultures prepared in the manner described by Uffredazzi are inadequate for a

quantitative study. The exact amount of material injected is unknown and the virulence of the bacteria may vary.

The strains of *M. tuberculosis* used in the study described below were obtained from the Trudeau Laboratory. Virulence was periodically checked, and the bacteria were recultured at intervals. Exact amounts of tubercle bacilli were used, measured in milligrams. All experiments were performed both with human and bovine bacilli. It is well known that rabbits are sensitive to bovine and not to human tubercle bacilli and therefore the results vary, depending upon the type of microorganism used.

In the first experiments 18 rabbits were used. They were injected with human bacilli (strain H37Rv) as follows:

6 rabbits were injected with 1 mg. of human bacilli.
6 rabbits were injected with 2 mg. of human bacilli.
6 rabbits were injected with 4 mg. of human bacilli.

The injections were given subcapsularly and intracortically into the kidney substance under sodium pentothal anesthesia. Both kidneys were injected. Nephrectomies were performed according to the following schedule:

In 3 animals nephrectomy was performed three months after injection of the bacilli.
In 3 animals nephrectomy was performed four months after injection of the bacilli.
In 3 animals nephrectomy was performed five months after injection of the bacilli.
In 3 animals nephrectomy was performed six months after injection of the bacilli.

Six infected rabbits were maintained as controls. All of the animals were sacrificed at the end of eleven months. In spite of the fact that rabbits are not particularly susceptible to infection with human-type bacilli, in about a three-month interval after the injection a well defined pinpoint-sized or rice-sized tubercle developed at the site of injection.

Tuberculosis thus produced showed a spontaneous healing tendency and this healing was uninfluenced by surgery. The number of injected bacilli did not change the course of the disease. There was a hematogenous spread, in a few cases, which showed the same spontaneous healing as the renal disease. That the tuberculous changes were not due to foreign protein was shown by the fact that virulent tubercle bacilli were found in the focus for a long period. The tuberculous focus, however, had, as noted, a tendency to heal. Definite evidence of healing was seen not only by the disappearance of the tubercle bacilli from the site of lesion in a few months, but histologic studies showed a progressive healing of the lesion. The end result was a small fibrotic area in which the original tuberculous changes were no longer visible.

Thus this first series did not indicate that healing is faster in nephrectomized animals than in the control group.

In the next series, known amounts of standard virulent bovine bacilli (Ravenel) were used. Eighteen rabbits were injected as follows:

6 rabbits were injected with 0.2 mg. bovine bacilli in each kidney
6 rabbits were injected with 0.4 mg. bovine bacilli in each kidney
6 rabbits were injected with 0.8 mg. bovine bacilli in each kidney

The kidneys were injected subcapsularly and intracortically as in the pre-

vious experiment. There was a rapid hematogenous spread with involvement of practically all organs. The animals died before nephrectomies could be performed according to the schedule. In a few unselected cases, nephrectomies were performed at irregular intervals, but none of the nephrectomized rabbits survived surgery longer than two weeks.

The same experiment was repeated, using smaller and smaller amounts of tubercle bacilli. In the third series, 12 rabbits were used and each kidney was injected with 1:100,000 mg. dilution of culture of bacilli. In the fourth series, 12 rabbits were used and each kidney was injected with 1:500,000 mg. dilution of culture of bacilli. The result obtained was the same as in the second series, i.e., rapid hematogenous spread with involvement of almost all organs. In both groups several nephrectomies were performed, again at irregular intervals, because of the rapid death rate. The progression of the disease was uninfluenced by surgery. Evidently even a minimal number of bacilli caused widespread hematogenous spread. In the last series (4 rabbits), the right kidney was injected with 0.2 mg. of bovine bacilli intracortically and subcapsularly and the left kidney was removed at the time of injection. In this way an attempt was made to eliminate the unlikely possibility that special sensitivity of a paired organ increases the severity of the local infection. Nevertheless, the result obtained was the same as in any of the previous groups, i.e., hematogenous spread with massive involvement of all the organs. The progression of the disease in the solitary kidney was uninfluenced by the nephrectomy.

In all of these groups, almost every organ revealed some healing tendency, but the infection was so massive that no actual healing of any organ could have taken place. No evidence was obtained from this study that removal of a kidney will improve or help the condition of the remaining kidney. Moreover, there was no evidence that an involved kidney might cause such sensitivity in the opposite kidney that the removal of this kidney would be indicated.

Comment: As a result of experiments, no support was obtained for the theory that nephrectomy of the more diseased kidney will aid in the healing of the remaining infected kidney. Moreover, it appears that, within the limits of the infecting doses used in these studies, the number of bacilli injected does not play an important role in the pathogenesis of the disease. The severity of the disease depended upon the type of bacilli (human or bovine) and on the extent of the hematogenous spread. The resistance of the infected animal may play an important role in healing.

What of the role of the special sensitivity of paired organs? If a damaged renal tissue produces antigen against which antibodies are formed, and the antibodies would act upon an uninvolved side, then we would be dealing with a unilateral disease. However, the original hematogenous spread is bilateral and one side usually heals. When the diagnosis of bilateral renal tuberculosis is made after nephrectomy, the remaining kidney has already been diseased and the progression of the disease does not depend on the previously formed antibodies. If damaged renal tissue can act as an antigen, a solitary kidney with tuberculosis could also stimulate the production of antibodies.

The same objection may be pertinent in explaining contralateral disease on the basis of the Forssner experiment. Tubercle bacilli of a diseased kidney are potentially able to infect the opposite side. That is true, but infection on the opposite side is present prior to nephrectomy as a result of a hematogenous spread. Once the disease is bilateral, the Forssner experiment cannot be applied to indicate the validity of nephrectomy in bilateral disease.

Clinical Studies

Clinical studies on the service at Sea View Hospital supplement the data noted above. A study has been made of 57 patients upon whom nephrectomy for tuberculosis was performed, and in whom the preoperative diagnosis was predominantly unilateral disease; only the postoperative course showed the bilateral nature of the disease. The disease was considered bilateral if renal tuberculosis was present on both sides at the time of operation or if the second kidney showed involvement within eighteen months postoperatively.

In these 57 cases, disease in the remaining kidney appeared in the following way:

Cases studied with contralateral disease	57 patients
Appearance of disease occurred within six months	39 patients
Appearance of disease occurred within twelve months	53 patients
Appearance of disease occurred within eighteen months	56 patients
Appearance of disease occurred within twenty-four months	57 patients

The majority of these cases were not operated on at Sea View Hospital, but were transferred there after the disease in the remaining kidney had become apparent. These figures should serve as a constant warning of the danger of subsequent contralateral disease. Prognosis should always be guarded for a period of at least twelve to eighteen months, during which time the patients should have systemic treatment for tuberculosis. A special effort was made to study the progress of disease in the above group of 57 patients. Attempts were made to find evidence of healing on roentgenographic studies. There were 8 patients in whom the kidney disease seemed to improve for a while insofar as roentgenograms were concerned; but, nevertheless, the ultimate result was a steady progression of the disease. Evidence of healing was not greater in this group of patients than in those bilateral cases where no nephrectomy was performed. These 57 patients have not been followed long enough at present to determine the ultimate case mortality rate for the group.

In order to compare the result of surgically treated patients with those treated conservatively, a study was made of 69 patients with bilateral renal tuberculosis in whom conservative therapy was used. The duration of life of these patients was as follows:

9 dead within six months after the diagnosis was made
16 dead within twelve months after the diagnosis was made
39 dead within twenty-four months after the diagnosis was made
56 dead within thirty-eight months after the diagnosis was made

60 dead within forty-two months after the diagnosis was made
9 alive four years post diagnosis.

Although we have not had the opportunity to study the case mortality rate of the majority of the cases in which nephrectomy was done, the progressive, rather rapid deterioration of the majority of patients who had surgery clearly indicated that nephrectomy did not offer significantly better results than conservative therapy in these cases.

DISCUSSION

These studies, both the experimental and clinical, thus fail to reveal any evidence that nephrectomy in cases of bilateral disease has any ameliorating effect on the remaining kidney. Nephrectomy did not apparently promote a healing tendency in the remaining kidney.

The survival period of a patient after nephrectomy depends upon the amount of remaining functioning kidney tissue. If disease is fairly well advanced, bilaterally, the postoperative survival period is short. In 12 cases in the present series in which the remaining kidney showed a fair amount of destruction within six weeks postoperatively, no patient lived longer than eighteen months. Without operation, similar pathologic changes are associated with a survival period of at least that length.

There are, however, cases in which nephrectomy must be done despite the bilateral nature of the disease. If pyonephrosis is present, with high temperature which cannot be controlled by conservative measures, nephrectomy has to be done. Uncontrollable bleeding is also a definite indication for removal of the diseased kidney. In an occasional case the pain is so severe, because of repeated colics, that nephrectomy is advocated. In all of these situations, however, the nephrectomy is not performed to improve the condition of the remaining kidney, but to cope with an immediate emergency.

In cases of apparently unilateral tuberculosis where roentgenographic evidence of the disease is present or where clinical symptoms are present, the best therapy is nephrectomy, as these cases seldom heal. If only a minimal lesion is present, the operation should be performed only after a certain period of observation and conservative therapy. In some such cases healing may be expected. On the other hand, if there is evidence of progression of disease, operation is indicated. Bacilluria per se is not an indication for operation.

The average ten-year survival rate of patients with unilateral involvement, after nephrectomy, as reported from various clinics, is over 50 per cent. However, this is not the case if surgical treatment is withheld. Parsons (17) showed that 85 per cent of medically treated patients with unilateral kidney tuberculosis were dead in five years. Wildbolz (5), in 316 nonoperated cases, showed a 58 per cent five-year case mortality and only six per cent were alive after ten years. On the other hand, of 104 patients on whom he performed nephrectomies for tuberculosis, 55.7 per cent were well at the end of ten years and 59 per cent were alive. Of 863 cases (18) operated on at the Mayo Clinic, 58.1 per cent were

clinically well four years after operation. Of 260 cases analyzed by Nesbit, Keitzer, and Lynn (19), 50.3 per cent of patients who had nephrectomies for tuberculosis were living and well eleven years after operation. Emmett and Kibler (20) found that if urine from the remaining kidney was negative after nephrectomy, the five-year mortality rate was 20.3 per cent and for ten years, 34 per cent. If pus cells were present, the mortality rate was 40 per cent and if bacilluria were found in addition to pus cells, the five-year case mortality rate was 60 per cent.

SUMMARY AND CONCLUSIONS

On the basis of experiments in rabbits and study of 126 patients with renal tuberculosis, no evidence was obtained to support the concept that nephrectomy increases the healing tendency of the remaining kidney in bilateral renal tuberculosis.

The prognosis in renal tuberculosis should be guarded for at least eighteen months after nephrectomy, as the apparently uninvolved side might contain silent tuberculous foci which progress to the point of recognition postoperatively. These occasional instances of renewed appearance of disease postoperatively are not contraindications to nephrectomy, for the best available therapy for unilateral tuberculosis of the kidney is nephrectomy.

Spontaneous healing in early cases, mostly those without roentgenographic evidence of the disease, can occur. Bacilluria with *M. tuberculosis* per se is no indication for surgical intervention.

SUMARIO AND CONCLUSIONES

La Patogenia y el Pronóstico de la Tuberculosis Renal

No hay pruebas de que la nefrectomía realce la tendencia curativa del riñón restante en la tuberculosis renal bilateral.

El pronóstico debe ser reservado por lo menos por dieciocho meses después de la operación, pues el lado aparentemente indemne tal vez contenga focos tuberculosos latentes que pueden desintegrarse postoperatoriamente. Esas desintegraciones ocasionales no constituyen contraindicaciones de la nefrectomía, dado que ésta representa la mejor terapéutica para la tuberculosis unilateral del riñón

Puede sobrevenir curación espontánea en los casos tempranos, principalmente, sin signos radiográficos de la dolencia. La baciluria tuberculosa no constituye, en sí misma, indicación de la intervención cruenta.

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THE STREPTOMYCIN TREATMENT OF GENITOURINARY TUBERCULOSIS[1,2,3,4]

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INTRODUCTION

Two hundred and fifty-three cases of genitourinary tuberculosis have now been treated with streptomycin alone by the Veterans Administration, Army, and Navy Research Group. These patients were studied exhaustively. Nine consecutive urine cultures for tubercle bacilli were made every month during treatment and every three months thereafter. The patients were cystoscoped before and after treatment and every six months for a period of two years. The findings were recorded graphically each time for comparison (figure 1). Retrograde and intravenous pyelograms were made on these occasions. Kidney function tests, blood counts, caloric and audiographic tests were done every two weeks during treatment, and daily observations were made for symptoms and possible toxic manifestations. Patients with all types and all stages of tuberculosis were treated. It was recognized that the genitourinary tuberculosis was only one complication of a generalized tuberculous infection which usually originated in the lungs and spread by the hematogenous route to the genitourinary organs, the kidneys, the prostate, and the epididymides. It was further recognized that when active tuberculosis was found in one kidney, it was necessary to suspect that there were dormant tubercles in the other kidney which might later become active if resistance was lowered. Active tuberculous lesions in the kidneys appeared to destroy them by the formation of strictures, ulcerations, and excavations.

DOSAGE

The most effective dosage regimen against active, ulcerating kidney lesions, when streptomycin alone was used, appeared to be intramuscular injections of 0.3 Gm. every four hours, day and night, for 120 days. Tubercle bacilli disappeared at once from the urine of 83 per cent of 64 patients on this regimen, and at the end of six months urine specimens from 68 per cent of the original

[1] From the Veterans Administration Hospital, Bronx, New York, New York.

[2] Presented before the Medical Section, as part of the symposium on *Streptomycin and Extrapulmonary Tuberculosis*, at the 45th annual meeting of the National Tuberculosis Association, Detroit, Michigan, May 4, 1949.

[3] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[4] This study is one part of a coordinated large-scale program of investigation into the effects of streptomycin on tuberculosis of all types now being conducted by the Veterans Administration, the Army, and Navy. Since their veteran patients will be followed indefinitely by the Veterans Administration Hospitals, they present an unparalleled opportunity for long-term follow-up.

group were still negative for bacilli. If the dosage or length of treatment were lessened, the effectiveness of the drug decreased. When 0.5 Gm. of streptomycin was given every twelve hours for 120 days to 42 patients, the excretion of *M. tuberculosis* in the urine ceased immediately in 60 to 75 per cent of the group, but six months later in only 46 per cent of the group were the urines negative for tubercle bacilli. Forty-two-day—treatment regimens did not appear to eliminate the organisms from the urine as satisfactorily. It was found that the smaller the lesions were, the more effective was the streptomycin treatment. The topical effect of a continuous urinary level appeared to be beneficial for bladder lesions (figure 2).

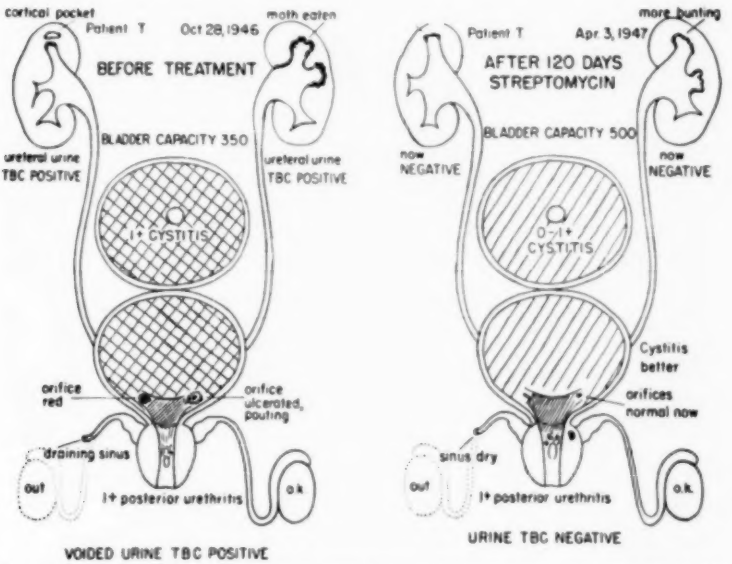

FIG. 1. Cystoscopic findings were recorded graphically on these charts every three months for comparison.

LARGE KIDNEY LESIONS

Large kidney lesions responded poorly. Most patients with kidney tuberculosis were not discovered until their lesions were so large that they were definitely visible in the pyelogram. Any lesion which was visible in the pyelogram was assumed to contain necrotic and fibrous tissue and was, therefore, classified as a "large" kidney lesion even if only a single calyx of the kidney was involved (figure 3B). In one group of such patients, followed for eighteen months, tubercle bacilli disappeared from the urine in about 25 per cent of the cases after the 2-gram–120-day treatment, although in many additional patients progress of the disease was apparently slowed. It was apparent that if such a large kidney

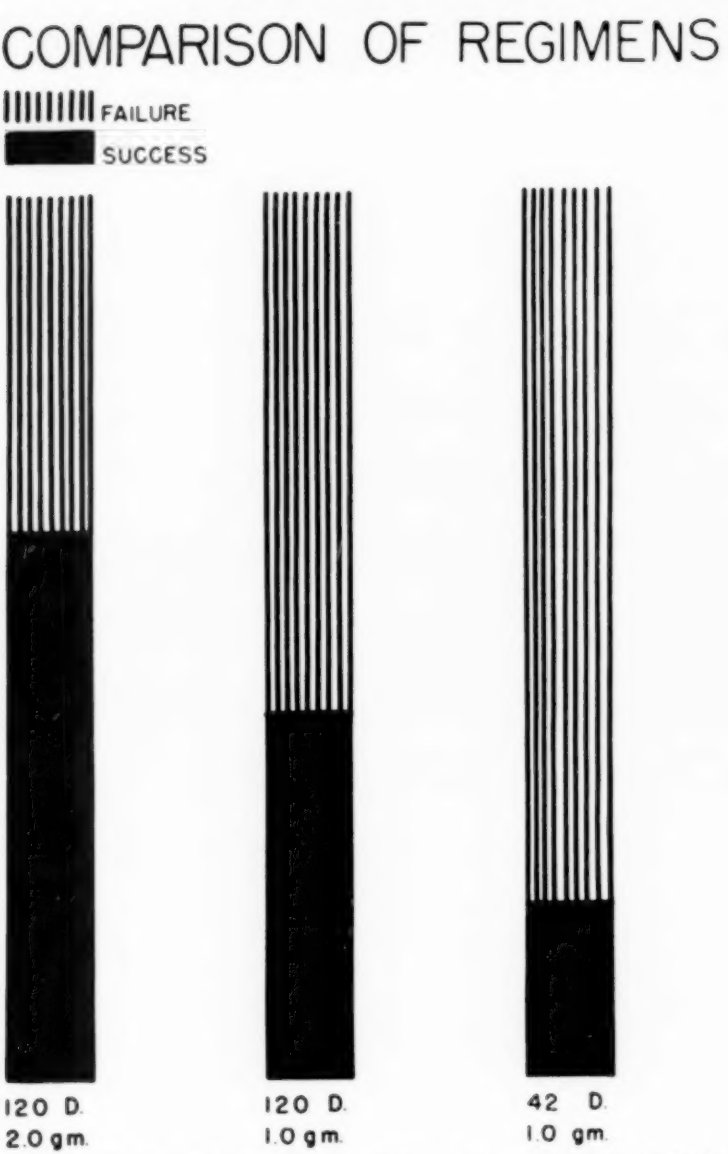

FIG. 2. The most successful dosage regimen (left-hand column) was 2 Gm. for 120 days. Sixty-two of those cases have been negative for over one year. All other regimens have been less effective.

lesion was present in only one kidney, while the other kidney showed no activity, surgery was preferable to streptomycin treatment. Six months of bed rest was advised following surgery, and it was recommended that a very careful watch be kept over the remaining "good" kidney to detect any signs of early breakdown of the tubercles which might be present. It is said that before the development of

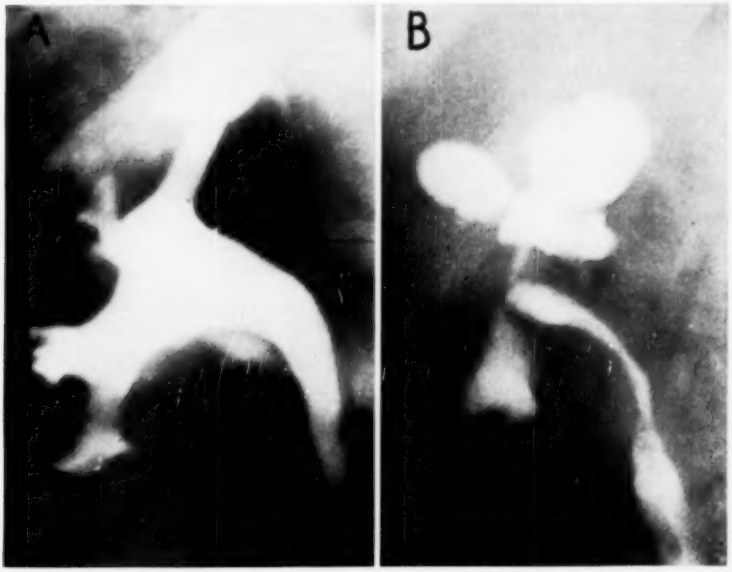

FIG. 3. Kidney "A" (left) is an example of an actively tuberculous kidney with pus and tubercle bacilli in the ureteral urine. The lesions are so small, however, that they are not visible in the pyelogram. Such lesions responded well. Kidney "B" (right) shows an example of a "large" kidney lesion with strictures and necrotic tissue. Streptomycin checked few such cases.

antimicrobial therapy only about 50 per cent of these patients lived more than five years following the removal of a tuberculous kidney.

SMALL KIDNEY LESIONS

"Small" kidney lesions responded well following streptomycin therapy. A "small" tuberculous lesion was one which was discovered by means of bacteriologic examination of urine before there was a visible pyelographic lesion, and probably contained relatively little necrosis and fibrous tissue (figure 3A). In one pilot series with such renal lesions, the urines have become consistently negative for tubercle bacilli for a two-year period on 8 of 10 patients, and the urines of the other two patients are probably negative. Unfortunately, patients with "small" kidney lesions are almost never seen by the practicing urologist.

They may be found by the urologist who removes a tuberculous kidney and then watches the other "good" kidney for the first sign of disease which might appear as pyuria or the excretion of tubercle bacilli in the urine. The early ("small") renal lesions may also be found by the physician who specializes in pulmonary tuberculosis and who watches his patients carefully for the appearance of pyuria, albuminuria, or tubercle bacilli in the urine. A periodic urinalysis should certainly be obtained for every patient who has tuberculosis in any part of the body.

CYSTITIS AND URETERITIS

Superficial ulcerations and edema of the bladder and ureters responded well to streptomycin treatment (figure 4). Contracted bladders and strictures of the

FIG. 4. Narrowed area in the left lower ureter which disappeared during streptomycin treatment. Occasionally, however, such strictures became much worse during streptomycin treatment.

ureter due to deep-seated fibrous lesions did not improve. Streptomycin treatment did appear to hasten fibrosis of the ureters and bladder in rare cases.

PROSTATIC AND EPIDIDYMAL TUBERCULOSIS

Prostatic and epididymal tuberculosis did not respond sufficiently well to merit treatment with streptomycin except where severe cystitis has resulted from local extension on to the bladder floor adjacent to the prostatic urethra. Epididymal tuberculosis was preferably treated by surgery, accompanied by three weeks of streptomycin treatment.

Surgery and Streptomycin: Surgery was preferable to streptomycin for large necrotic tuberculous lesions of the kidney or epididymis. A three weeks' course of streptomycin, 0.5 Gm. every twelve hours, was given at the time of operation upon any tuberculous organ. It was recognized that this prophylactic treatment did not protect the patient against a tuberculous wound infection if live tubercle bacilli were spilled into the wound. It was hoped, however, that the three weeks of streptomycin would help to prevent hematogenous dissemination from the operative manipulation.

TOXICITY

Some degree of permanent damage to the vestibular apparatus followed the 2-gram–120-day regimen in all patients. With corrective exercises, however, the

patients compensated adequately for this loss. Deafness was not a problem on this dosage. Slight transitory rises in the nonprotein nitrogen concentration in the blood were observed in a few patients but renal toxicity was not impressive. Even the patients with only a fragment of a solitary kidney remaining tolerated the drug well. Dermatitis occurred following the use of some of the early lots of streptomycin.

LIMITATIONS OF STREPTOMYCIN

The development of bacterial resistance to the drug and the presence of necrotic, caseous, or fibrous tissue were the chief factors limiting the effectiveness of streptomycin. If treatment was started early enough, the results were excellent. It was obvious that, if the patient had only one kidney or if both kidneys were involved, streptomycin was the treatment of choice. Combined therapy employing streptomycin and para-aminosalicylic acid is now under investigation.

CONCLUSION

Streptomycin alone was sufficiently effective to be useful against bilateral, solitary, and selected small unilateral tuberculous kidney lesions. Small kidney lesions responded better than large lesions. The most effective dosage regimen was 0.3 Gm. every 4 hours, for 120 days by the intramuscular route. Even patients whose urines remained positive for tubercle bacilli were often improved clinically. It is now hoped that toxicity can be reduced and effectiveness increased by combined treatment with other tuberculostatic drugs such as para-aminosalicylic acid.

CONCLUSIONES

La Estreptomicinoterapia de la Tuberculosis Genitourinaria

La estreptomicina sola resultó suficientemente eficaz para mostrarse útil contra las lesiones tuberculosas bilaterales, solitarias y ciertas pequeñas unilaterales del riñón. Las pequeñas lesiones renales respondieron mejor que las grandes. El régimen posológico más efectivo fué de 0.3 Gm. cada cuatro horas, durante 120 días. Aun los enfermos cuyas orinas continuaban siendo positivas mejoraron a menudo clínicamente. Espérase ahora reducir la toxicidad y acrecentar la efectividad mediante el tratamiento combinado con otras drogas tuberculostáticas, tales como el ácido para-amino-salicílico.

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THE EFFECT OF STREPTOMYCIN ON THE MORPHOLOGY OF THE TUBERCULOUS LESION[1]

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INTRODUCTION AND REVIEW OF THE LITERATURE

The use of streptomycin in the treatment of active tuberculosis has altered the former clinical course, prognosis, and expected results of treatment. In the majority of cases where definite indications for the use of the drug exist, clinical and roentgenographic modification of the tuberculous lesions can be demonstrated. The purpose of this communication is to present the effects of the drug on the morphology of the lesions at autopsy, and to compare some of the findings with those observed in identical forms of tuberculosis coming to autopsy before streptomycin was available.

Recently, several observers have reported the pathological changes produced in the tuberculous lesions of streptomycin-treated cases. Baggenstoss, Feldman, and Hinshaw (1) first presented 5 cases of miliary and meningeal tuberculosis which had received streptomycin during life. Autopsy examination of the organs of these patients revealed definite evidence of regression and healing in the miliary tubercles of the lungs, liver, and spleen. The criteria of healing included fibrosis, hyalinization, and lack of caseation in the miliary tubercles. Moreover, regressive change did not appear to follow the usual pattern of encapsulation of a cellular or caseous central mass, but represented, rather, a diffuse, even fibrosis of the entire lesion. These authors noted that, under streptomycin administration, tuberculous lesions may heal in one or more organs, while active progression occurs in others. This phenomenon was thought to be due to several factors: (*1*) location of the organ; (*2*) circulation of the blood and lymph; (*3*) anatomic and histologic structure of the organ; (*4*) native resistance of certain organs (the kidneys, adrenals, prostate, and brain are involved only slightly in miliary tuberculosis); (*5*) size or mass of lesion at the time when streptomycin therapy was begun; (*6*) concentration of streptomycin in fluids and tissues after its parenteral administration. This last observation was verified by post-mortem examination and determination of streptomycin concentrations in experimental animals which had received measured doses of the drug immediately prior to death.

Several months later, Kidd (2) reported 7 fatal cases of streptomycin-treated miliary tuberculosis from the New York Hospital; all had detailed autopsy study. Many organs, especially the lungs, liver, spleen, and kidneys, showed definite evidence of healing of tuberculous foci. In addition, however, 5 of these cases showed pathological evidence of fresh tuberculous lesions whose histologic structure indicated that they had appeared after the streptomycin administration had been initiated. Often healed lesions and active ones were present side by side in the same microscopic field. Review of the clinical and bacteriological course in these individuals indicated a definite coincidence between clinical relapse, the bacteriological demonstration of drug-resistant bacilli, and the pathological presence of fresh tuberculous lesions which had developed during the course of administration of the antibiotic.

The striking diminution in perifocal reaction around pulmonary tuberculous lesions in

[1] From the Laboratories of Triboro Hospital and Queens General Hospital, Jamaica, Long Island, N. Y.

patients who had been treated with streptomycin is stressed by Auerbach and Stemmermann (3) as the most readily observed change. In a report of 7 such cases, they were impressed by the relatively empty alveolar spaces surrounding the focal lesions in the lung. This is clinically manifest by the clearing of the parenchymal infiltrate on serial roentgenograms. Thus, the nonspecific capsule, usually seen about tuberculous lesions as a result of organization and fibrosis of the perifocal exudate, was either markedly or entirely absent in those patients who had been given streptomycin prior to death.

Flory, Correll, *et al.* (4) reported further histological observations in the 7 cases of streptomycin-treated tuberculosis autopsied at the New York Hospital and previously presented. Again definite alterations in the direction of healing of the tuberculous foci were noted. This varied from focal fibrotic scars containing a few macrophages and lymphocytes without specific evidence of pre-existing tuberculosis to apparent exaggerations of the collagen which normally surrounds the caseous centers of miliary tubercles. They concluded that definite evidence of healing can be demonstrated in miliary tubercles and in miliary tuberculosis; further, that such healing could not be found in a single instance in reviewing 20 cases of tuberculosis which had received no streptomycin. Caseous lesions and cavity walls were not apparently affected by administration of the drug.

In the light of these previous observations, all indicative of a partial, but definite, effect of streptomycin on the histology of the tuberculous lesion, we have sought to analyze the anatomic changes produced by the use of the drug in cases of hematogenous and pulmonary tuberculosis which have come under review in the past 15 months. The criteria of healing and inactivity used were similar to those employed in the studies mentioned.

MATERIAL

The clinical histories, gross and microscopic findings in twenty cases of streptomycin-treated tuberculosis autopsied at Queens General and Triboro Hospitals, were reviewed. The protocols were chosen consecutively, irrespective of age, race, type of tuberculosis, total streptomycin administered, or the stage of the disease during which the streptomycin was administered.

The patients ranged from six months to 53 years in age; 10 were males, 10 females. Three Negroes are included in the series. The total amount of streptomycin given ranged from 12 Gm. to 218 Gm. Two of the cases received only 12 Gm. and 13 Gm., respectively, but these were included for completeness. The drug was given in divided doses by the intramuscular route; only occasionally was the intrathecal route used. The dose for adults was generally 1 Gm. daily; in an unusual instance, 0.5 Gm. or 2 Gm. daily were used. The 3 children included in the series received smaller daily doses proportionate to their body weight.

In an attempt to interpret the efficacy of streptomycin, the time intervals between onset of illness to the first dose and the time interval from the last dose to death are charted. In most cases the patients were maintained on conservative measures for a considerable time before streptomycin was introduced. The children presented much more definite indications for the use of the drug, and hence received the antibiotic at a relatively earlier stage of their disease.

For purposes of comparison, a group of autopsied cases of hematogenous and pulmonary tuberculosis were reviewed in which no streptomycin had been given.

It was attempted to choose approximately parallel cases for the two groups studied so that all of the factors would be similar except for the fact that the 20 reported cases had received streptomycin during the course of the disease.

OBSERVATIONS

In order to facilitate the formation of impressions from a study of the material at hand, the cases have been classified according to the general type of clinical tuberculosis which they represented. Three groups are thus included: (*1*) primary tuberculosis, 3 cases; (*2*) hematogenous tuberculosis, 6 cases; (*3*) chronic pulmonary tuberculosis, 11 cases.

PRIMARY TUBERCULOSIS (Table 1)

The most striking and most dramatic gross and microscopic pathological changes were seen in the 3 cases of tuberculosis in children who had received streptomycin therapy during all or the major portion of their clinical course.

Primary complex: In one of the cases (Case 3), the Ghon focus was determined with reasonable certainty. Microscopic sections of this area showed scarred healing without caseation or calcification (figure 1). This uncommon mode of healing could well be a noteworthy effect of the streptomycin therapy. The hilar lymph nodes of both Case 2 and Case 3 presented regression of the tuberculosis with calcification.

Miliary dissemination: There was extensive healing of the tuberculous lesions in the lungs in all three cases of miliary dissemination (figures 2a, b, c, d). In Cases 2 and 3, no evidence of any active lesion was found. The complete healing of the small miliary foci in the various organs has been emphasized by previous writers (1) and was well demonstrated in all 3 cases (figures 3a, b; 4; 5; 6 a, b).

Brain: Tuberculomas, with persistent caseation and a wall of granulation tissue, were noted in Case 1 (figure 7a); the overlying meninges showed fibrosis. In Case 2, the one focus noted in the dorsum of the cerebrum was made up solely of a nonspecific granulation tissue (figure 7b), and was contiguous with the overlying fibrosed meninges. Clinical evidence pointed to the existence of a cerebral lesion at this site almost at the very onset of the patient's disease. This would favor a localized tuberculoma rather than a focal tuberculous meningitis at such an early date.

The prolongation of the course of the disease by streptomycin therapy, with the increase in degree of meningeal fibrosis and vascular changes, has caused the more frequent appearance of the complications of hydrocephalus and encephalomalacia.

Meninges: Healing in the meninges, characterized by fibrosis, was present in all 3 cases and to a degree considerably beyond that expected in the clinical course usually associated with this age group.

Vessels of the meninges: The intimal hyperplasia of the arteries in the meninges, pointed out by Flory, *et al.*, was seen in Cases 2 and 3 (figures 8 a, b, c). Multiple sections of the same vessel showed the intimal hyperplasia to be focal.

Dilatation of terminal respiratory units, with small cyst formation, was marked

TABLE 1

Cases of Primary Tuberculosis

CASE NUMBER	AGE	SEX	RACE	CLINICAL DATA	TOTAL STREPTOMYCIN	TIME INTERVAL			PRIMARY COMPLEX		AUTOPSY FINDINGS					MENINGES	MENINGEAL VESSELS
						Total illness	Onset to first dose	Last dose to death	Lung	Regional nodes	Miliary dissemination		Brain				
											Lungs	Other organs	Tuberculoma	Hydrocephalus	Encephalomalacia		
					Gm.	*months*	*weeks*										
1	6½ months	f	c	Admitted with one-week history of cough, fever, severe dyspnea. X-ray: miliary dissemination in lungs. Cerebrospinal fluid, negative.	64	5	3	0	Organizing tuberculous pneumonia, LLL	Large caseous	Active and healed	Active and healed in liver, spleen, kidneys, bone marrow	1 cm. caseous focus, thalamus; 2 mm. caseous focus, frontal lobe	0	0	Regional fibrosis overlying tuberculoma	Negative
2	2 years	f	w	Admitted with history of limp for 6 weeks. Spiking temperature with remissions. Tuberculous meningitis.	123	6	2 to 3	0	Scant, diffuse fibrosis, LLL; no definite Ghon focus	Small caseo-calcific	Healed	Healed in liver	1 cm. focus, left cerebral cortex, made up of non-specific granulation tissue	Marked	0	Active caseous and fibrosis; regional fibrosis overlying cerebral focus	Intimal hyperplasia of medium-sized and small arteries
3	16 months	f	w	Admitted with left-sided weakness and spasm, left arm and left leg, of short duration. X-ray: infiltration, right mid-lung. Tuberculous meningitis.	72	6	6	0	2 mm. subpleural scar, RLL, interpreted as healed Ghon focus	Large caseous, early calcific	Healed	Active and healed in spleen; healed in bone marrow	0	Mild	Basal ganglia, bilateral and right internal capsule	Active caseous and fibrosis	Intimal hyperplasia of arteries

in Case 1 (figure 9a). These cysts showed no inflammatory infiltration in their walls and could best be interpreted as acquired, concomitant with the severe dyspnea during the prolonged course of the disease. Case 3 also showed dilated bronchioli lying in the fibrosed lung (figure 9b).

For comparison, 4 cases of primary tuberculosis autopsied in the last few years were reviewed. These had not been given streptomycin. The age range of the patients was from seventeen months to four years. In all, the primary complex was caseous; in one, partly calcific. In one case, the lung, in addition to fresh dissemination, showed some diffuse scarring. Several showed patchy atelectasis. All showed miliary dissemination. One had healed, hyalinized, spherical miliary tubercles in the lungs. None had complete healing of the miliary dissemination in the organs.

In one case, intimal hyperplasia of the meningeal vessels was found (figure 10); this was not as marked as that noted in the streptomycin-treated cases (figures 8a, b, c). Moderate hydrocephalus was found in 2 cases. Areas of cerebral softening were seen grossly in one case and microscopically in another case.

Primary tuberculosis treated with streptomycin, therefore, shows morphologic changes which can be attributed to an effect of the drug. The essential difference is a quantitative one; the streptomycin enhances the healing processes and brings the lesions to an end-stage of fibrosis seen less frequently in the cases of primary tuberculosis during the pre-streptomycin era.

HEMATOGENOUS TUBERCULOSIS (Table 2)

Six of the present series of cases were included in the category of hematogenous tuberculosis. The morphologic changes seen in the meninges in these patients were dramatic and worthy of emphasis.

Hematogenous dissemination: The miliary lesions showed a degree of healing similar to that observed in the cases of primary tuberculosis in children. Focal scarring was noted in many areas of the lungs. Tubercles in the liver and spleen were, for the most part, fibrotic (figure 11a). Figure 11b shows a healed focus in the testicle. There was one instance of healed pyelonephritis, present in Case 8 (figure 12). Definite tuberculous etiology could not be determined, but the renal scarring in the presence of other extrapulmonary tuberculosis strongly suggests a healed acid-fast process.

Brain: In addition to tuberculous caseous foci, areas of cerebral softening were noted in cases of Groups 1 and 2 (figure 13). The causal relationship of this softening was thought to be one of two possibilities: (*1*) the vascular lesions seen in the meninges; or (*2*) the marked fibrotic meningeal reaction with subsequent scarring and retraction producing sufficient distortion and compression of the regional vessels to interfere mechanically with the passage of blood. The presence of the most marked fibrosis is in the interpeduncular space with extension over the large sulci following the course of the main cerebral vessels. The fact that the fibrosis can obstruct the ventricular outflow at the foramina of Magendie and Luschka is manifest by the finding of a degree of hydrocephalus in many of these cases. This observation has been previously confirmed.

TABLE 2

Cases of Hematogenous Tuberculosis

CASE NUMBER	AGE	SEX	RACE	CLINICAL DATA	TOTAL STREPTOMYCIN	TIME INTERVAL			LUNGS	EXTRAPULMONARY TUBERCULOUS FOCI	MILIARY DISSEMINATION	TUBERCULOMA	HYDROCEPHALUS	ENCEPHALOMALACIA	MENINGES	MENINGEAL VESSELS
						Total illness	Onset to first dose	Last dose to death								
	Years				*Gm.*	*Yrs.*										
4	40	m	w	Bilateral pulmonary tuberculosis; LUL cavity; tuberculous osteo, vertebra; tuberculous meningitis	93	8	7 years 7 months	0	Cavity, left apex encapsulated caseous foci; active miliary with exudative pneumonia	Caseous tuberculosis of prostate; adrenals; vertebrae; psoas abscess	Active in spleen, liver, kidneys; healed in liver, pancreas	0	Moderate	Right basal ganglia and internal capsule	Caseous, with fibrosis	Intimal hyperplasia, small and medium-sized
5	50	m	w	Bilateral pulmonary tuberculosis, collapse therapy; tuberculous meningitis	108	6	4 years 6 months	0	Cavity, RUL fibrocaseous, LUL	Fibrocaseous tuberculosis, prostate	Active, liver and spleen	0	0	0	Caseous	No change
6	52	m	w	Bilateral pulmonary tuberculosis; tuberculous laryngitis; tuberculous epididymitis; tuberculous meningitis	218	8	6 years	0	Fibrosis and cavity, RUL; proliferative and caseous dissemination	Caseous tuberculosis, prostate; hyalinized focus, left testicle	Active, liver, spleen, adrenals, kidneys	Small, caseous; foci of granulation tissue	0	0	Caseous with fibrosis	No change
7	42	m	w	Caseocavernous tuberculosis with cavity, LUL; tuberculous meningitis	86	1	7 months	0	LUL cavity; exudative bronchopneumonia	Fibrocaseous foci in kidneys; caseoproductive tuberculosis, prostate; epididymis	Healed in liver, spleen	0	0	Basal ganglia	Caseous with fibrosis	Intimal hyperplasia
8	47	f	w	Pulmonary tuberculosis, moderate; tuberculosis of both breasts; tuberculous meningitis	62	20	19 years 6 months	0	Fibrotic RUL cavity, apex RLL	Healed renal papillitis; tuberculous pyelonephritis	0	0	Moderate	0	Caseous with fibrosis	Intimal hyperplasia; small and medium-sized vessels
9	52	m	w	Bilateral pulmonary tuberculosis; tuberculosis of tongue and larynx; tuberculous epididymitis; tuberculous arthritis; hematogenous dissemination; amyloidosis	40	6	5 years	1 year	RUL and LUL fibrosis, cavitation and encapsulated foci	Caseous focus, prostate; fibrocaseous pyelitis with scarring	Active in liver, spleen, ¶ kidneys	Not examined				

Meninges: Some degree of healing was present in all cases of tuberculous meningeal involvement (figure 14). In one instance, Case 5, this was overshadowed by the extensive caseo-exudative reaction; this patient had received intermittent courses of streptomycin for one year before the onset of his meningitis. Hence, although not substantiated bacteriologically, it is quite probable that the bacilli had become streptomycin-resistant. The remaining 4 cases, however, showed much more evidence of a healing fibrotic basilar meningitis. Histologically, these meninges were thickened, with considerable numbers of lymphocytes. Occasional areas of epithelioid tubercles were seen, some with active caseation, but the predominance of reaction was toward a productive healing.

The vascular lesions seen in the meninges of tuberculous meningitis include: (*1*) arteritis or phlebitis; (*2*) arteritis or phlebitis with fresh or organized thrombosis (figure 15); (*3*) intimal hyperplasia (figures 16 a, b, c).

The intimal hyperplasia appears to be the effect of chronic infection. It is analagous to the intimal hyperplasia seen frequently in the arteries of other organs, such as lungs or kidneys, in the presence of specific or nonspecific chronic inflammation. A toxic irritation of the vessel walls by the drug, streptomycin, has never been shown.

In 5 of the cases, the extrapulmonary tuberculous foci, especially in the prostate, were caseous, and apparently uninfluenced by streptomycin therapy; these remained as the source of persistent dissemination. In these 5 cases, also, the lungs at autopsy showed caseous or exudative tuberculosis in spite of the fact that streptomycin had been administered up to the time of death.

Untreated cases: For purposes of comparison, 11 cases of post-primary hematogenous tuberculosis with tuberculous meningitis, autopsied in the past few years, were reviewed. None had been given streptomycin.

All showed an exudative and caseous meningitis, but 5 of them also showed fibrosis of the meninges. In one case the fibrosis was localized over a cerebral tuberculoma, exactly as seen in figure 7a, Case 1. Intimal hyperplasia was seen in only one of the cases. This intimal hyperplasia was present in the medium-sized and smaller arteries of the meninges, the latter as marked as that seen in figures 16a and b, Case 8. This was the only case, also, which presented a marked hydrocephalus.

Eight of the cases showed a hematogenous dissemination to liver and spleen as well as to other organs. In 2 of these, many of the tubercles were healed; in one instance, calcified. The complete healing was seen in the smaller tubercles and was in the form of complete scarring, not necessarily spherical, exactly as seen in the streptomycin-treated cases.

Comment

Thus, none of the lesions seen in the streptomycin-treated cases were peculiar to these cases alone. Streptomycin enhances healing, particularly in small lesions and in exudative reactions. The actual mode of action of the streptomycin in promoting healing in tuberculosis is unknown. The efficacy of the drug is probably

due to its bacteriostatic action on the tubercle bacillus, rather than to its influence on the tissues of the host. The inhibition of the bacteria probably affords the opportunity for the tissue reactions to proceed to fibrosis. It is difficult, however, to draw conclusions from autopsy material of the above nature. What is required is an adequate standardization of drug dosage, control of the time of administration, complete ante-mortem and post-mortem bacteriologic studies of the resistance of the organism to the drug in each case and in the various tissues of each case, and, finally, a correlation of these observations with the gross and histologic picture of the tuberculous lesions. Above all, there is always present the problem of evaluating the tissue reaction in the disease tuberculosis, where the tempo and mode of reaction vary tremendously from host to host and from tissue to tissue in the one host.

CHRONIC PULMONARY TUBERCULOSIS (Table 3)

Three patients who underwent lobar resections are included in the series (Cases 10, 11, and 12). All of these patients died from postoperative complications within a short period after operation. In both Cases 10 and 11, the tuberculous activity in the lungs was well controlled. The discrepancy in the total streptomycin given in each case (in the former, 97 Gm.; in the latter, 12 Gm.), however, is strong evidence against the fact that the disease control was due to the influence of the drug.

In Case 12, some healing of the tuberculous dissemination in the lungs was noted. Such areas showed tubercles containing an unusual number of large giant cells with fibrosis (figure 17). Flory, *et al.* (4) call this chronic tuberculous pneumonia. Healing of such character was not seen as a common occurrence in a review of autopsied cases of far advanced pulmonary tuberculosis without streptomycin therapy. It can occur in the latter group, however, as shown in one case where, as an incidental finding, a small subapical pulmonary cavity with a bronchogenic tuberculous dissemination was found. This dissemination showed the typical picture of "chronic tuberculous pneumonia" and was interpreted as a tissue response in an individual with considerable resistance to tuberculosis. The latter patient had never received streptomycin therapy.

The last 8 cases were instances of far advanced pulmonary tuberculosis with bronchogenic spread. In this group, the evidence of healing due to streptomycin therapy was the most difficult to evaluate. Healing and progression in pulmonary tuberculosis generally take place side by side, so that the evaluation of scarring, encapsulation of caseous foci, and diminution in size of cavities must be guarded. A chronic tuberculous pneumonia was noted at times (figures 17 and 18).

The absence of a perifocal reaction in the vicinity of tuberculous foci in the lungs of streptomycin-treated patients has been pointed out (3). This is impressive when seen, but it is not an ubiquitous finding. The presence of an exudative and caseous reaction was, in fact, a common occurrence.

The lack of demonstrable effect on cavity walls was noted, as has been previously observed by earlier workers. In all instances, cavity walls showed well-developed zones of necrosis surrounded by a layer of epithelioid cells and granula-

tion tissue. In fact, the wide inner zone of caseous debris lining the cavities in streptomycin cases may be worthy of mention.

GENERAL COMMENT AND SUMMARY

The ever increasing clinical use of streptomycin in tuberculosis has focused our attention on the possible morphological changes produced in tuberculous lesions by the administration of the drug. Twenty autopsied cases of patients who had received varying amounts of the drug during their clinical course were selected. Indications for use of the drug included primary tuberculosis, hematogenous tuberculosis, and the pre- and post-operative use of the drug in patients who were submitted to resectional surgery.

From these observations it is strongly indicated that streptomycin administration can alter the tempo and degree of healing of the tuberculous lesions provided the drug is given during the time when it is efficacious and before resistant bacilli can become predominant. No histological finding, such as intimal hyperplasia of the smaller meningeal arteries, or the chronic tuberculous pneumonia, or the fibrous scarring, was of sufficient individuality to be peculiar to the effects of the drug. All could be demonstrated in cases which had not been given streptomycin. True "ring" lesions showing reactivation of tubercles beyond zones of healing were not found in this series.

The essential impression derived from this study is that no single gross or histologic change noted in the appearance of tuberculous lesions can be solely ascribed to the use of streptomycin. The use of the drug does, however, produce healing in small, fresh lesions more rapidly and to a greater extent than previously visualized.

SUMARIO

El Efecto de la Estreptomicina sobre la Morfología de la Lesión Tuberculosa

El empleo clínico cada vez mayor de la estreptomicina en la tuberculosis ha hecho concentrar nuestra atención en las posibles alteraciones morfológicas producidas en las lesiones tuberculosas por la administración de la droga. Escogiéronse para estudio 20 casos de enfermos autopsiados que habían recibido var as cantidades de la droga durante su evolución clínica. Las indicaciones para empleo de la droga comprendían tuberculosis primaria, tuberculosis hematógena y uso pre- y postoperatorio en los enfermos sometidos a la resección quirúrgica.

Queda poderosamente indicado que la administración de estreptomicina puede alterar la velocidad e intensidad de la cicatrización de las lesiones tuberculosas, con tal que se administre la droga durante el período de tiempo en que es eficaz y antes de que cobren predominio los bacilos resistentes. Ningún hallazgo histológico, tal como la hiperplasia de la túnica íntima de las arterias más pequeñas, o la neumonía tuberculosa crónica o la cicatrización fibrosa, revistió suficiente caracterización para ser considerado como propio de los efectos de la droga, pudiendo ser observados todos en pacientes que no habían recibido estreptomicina. En esta serie, no se encontraron verdaderas lesiones "anulares" mostrando reactivación de los tubérculos más allá de las zonas de cicatrización.

TABLE 3

Chronic pulmonary tuberculosis

CASE NO.	AGE	SEX	RACE	CLINICAL DATA	TOTAL STREPTOMYCIN	TOTAL ILLNESS	ONSET TO FIRST DOSE	LAST DOSE TO DEATH	SURGICAL PATHOLOGY	AUTOPSY FINDINGS
	Years				*Gm.*					
10	43	f	w	Bilateral pulmonary tuberculosis; RUL, RML lobectomy (pre-streptomycin); RLL lobectomy (post-streptomycin) thoracoplasty; pericarditis.	97	3 years 2 months	3 years	0	RUL, RML: caseocalcific foci (pre-streptomycin); RLL: apical cavity (post-streptomycin)	Left lung: extensive old scarring; encapsulated caseocalcific foci. Cause of death: acute pericarditis, nontuberculous
11	52	m	w	RUL cavity; RUL lobectomy; death 2 days postoperatively	12	3 years 3 months	same	0	Cavitation and fibrosis	Atelectasis and mucous plugs in right bronchial tree; fresh 2 cm. cavity in RLL; small, delimited caseous foci with scarring, left lung
12	32	f	w	LUL cavity; LUL lobectomy (with streptomycin); death 8 days postoperatively	61	11 months	5 months	0	2 cm. cavity; encapsulated caseous foci; tuberculous bronchitis, active	LLL and RUL, old caseous foci with tuberculous bronchitis; active bronchogenic dissemination, proliferative, exudative, and caseous (figure 17)
13	41	f	c	Cavity, LUL; no change with streptomycin; committed suicide	108	18 months	6 months	6 months		LUL cavity and fibrosis; encapsulated caseous foci. LLL and RUL: bronchogenic dissemination, proliferative, exudative, and caseous; tuberculous enteritis and laryngitis (figure 18)
14	19	m	c	Cavity, LUL; no change with streptomycin	46	11 months	3 months	7 months		Bilateral cavitation, with exudative and caseous pneumonia. Tuberculous laryngitis and enteritis; terminal miliary dissemination to liver and spleen

15	41	m	w	Bilateral advanced pulmonary tuberculosis; collapse therapy; no change with streptomycin	42	11 years	10 years	9 months		Far advanced bilateral pulmonary tuberculosis with bronchogenic spread, proliferative, exudative, and caseous
16	17	f	w	Bilateral pulmonary tuberculosis; poor response to streptomycin	47	1 year 10 months	1 year 4 months	0		Far advanced bilateral pulmonary tuberculosis with bronchogenic spread, proliferative, exudative, and caseous
17	35	f	w	Bilateral advanced pulmonary tuberculosis; no response with streptomycin; resistant bacilli	84	8 years	6 years	2 months		Far advanced bilateral pulmonary tuberculosis; empyema; bronchogenic spread, proliferative, exudative, and caseous; amyloidosis
18	33	f	w	Bilateral far advanced pulmonary tuberculosis; Short course of streptomycin due to resistant bacilli	13	13 years	12 years 3 months	9 months		Far advanced pulmonary tuberculosis with bronchogenic spread, proliferative, exudative, and caseous. Tuberculous laryngitis and enteritis; terminal miliary dissemination to liver, spleen, kidneys; amyloidosis
19	24	m	w	Bilateral advanced pulmonary tuberculosis; Initial improvement with streptomycin, but bacilli developed resistance	84	9 years	8 years	6 months		Far advanced bilateral pulmonary tuberculosis with bronchogenic spread, proliferative, exudative, caseous. Tuberculous enteritis; amyloidosis
20	52	m	w	Bilateral advanced pulmonary tuberculosis; no change with streptomycin	53	12 years	11 years	1 year		Far advanced bilateral pulmonary tuberculosis with bronchogenic spread, proliferative, exudative, and caseous. Tuberculous enteritis; amyloidosis

La impresión fundamental derivada de este estudio es que no cabe imputar exclusivamente a la estreptomicina ninguna alteración macroscópica o histológica aislada. El empleo de la droga sí produce cicatrización en las lesiones recientes y pequeñas más rápidamente y en mayor grado que lo observado anteriormente.

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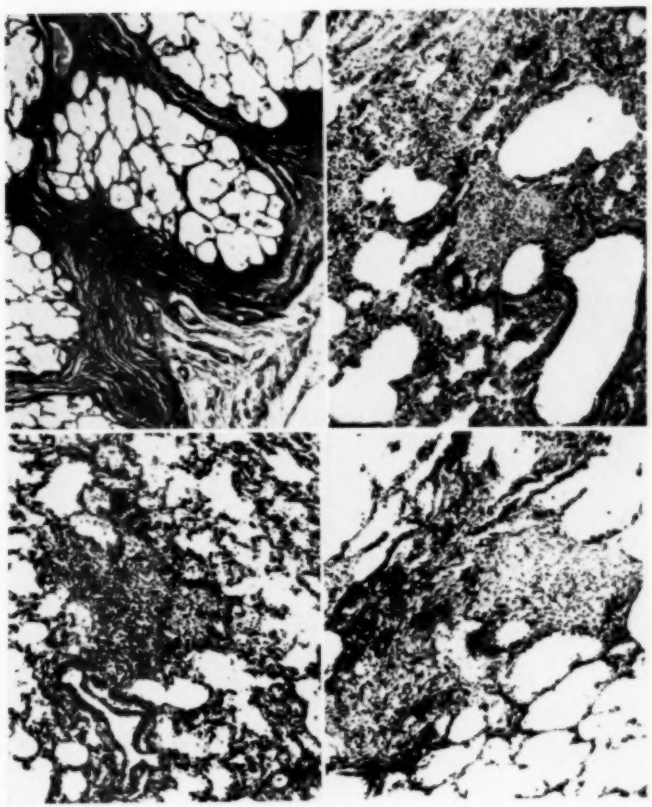


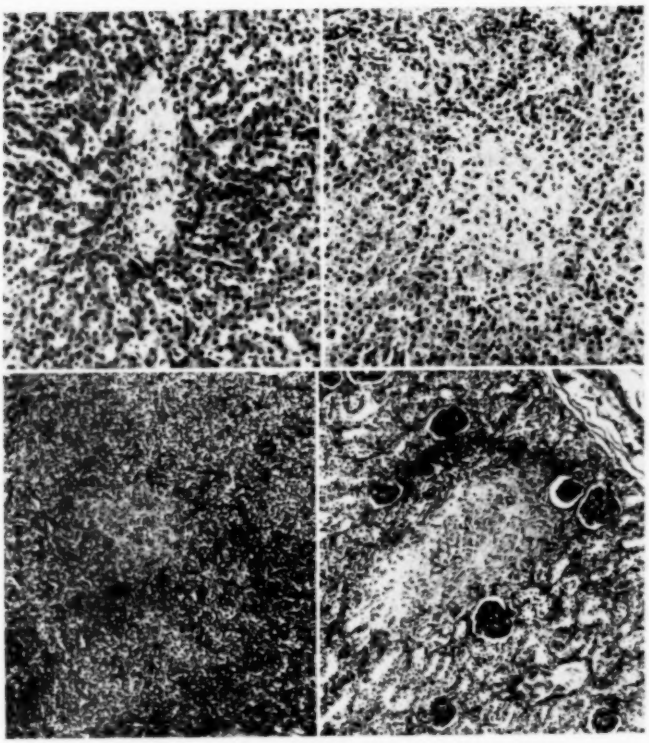
FIGS. 1–2c

FIG. 1. (Upper left) Case 3. Lung with healed subpleural primary focus, showing fibrosis without caseation or calcification. (Streptomycin-treated)

FIG. 2a. (Upper right) Case 1. Lung; miliary tubercles showing complete healing by diffuse, even fibrosis. (Streptomycin-treated)

FIG. 2b. (Lower left) Case 2. Lung; miliary tubercles showing complete healing by diffuse, even fibrosis. (Streptomycin-treated)

FIG. 2c. (Lower right) Case 3. Lung; miliary tubercles showing complete healing by diffuse, even fibrosis. (Streptomycin-treated)



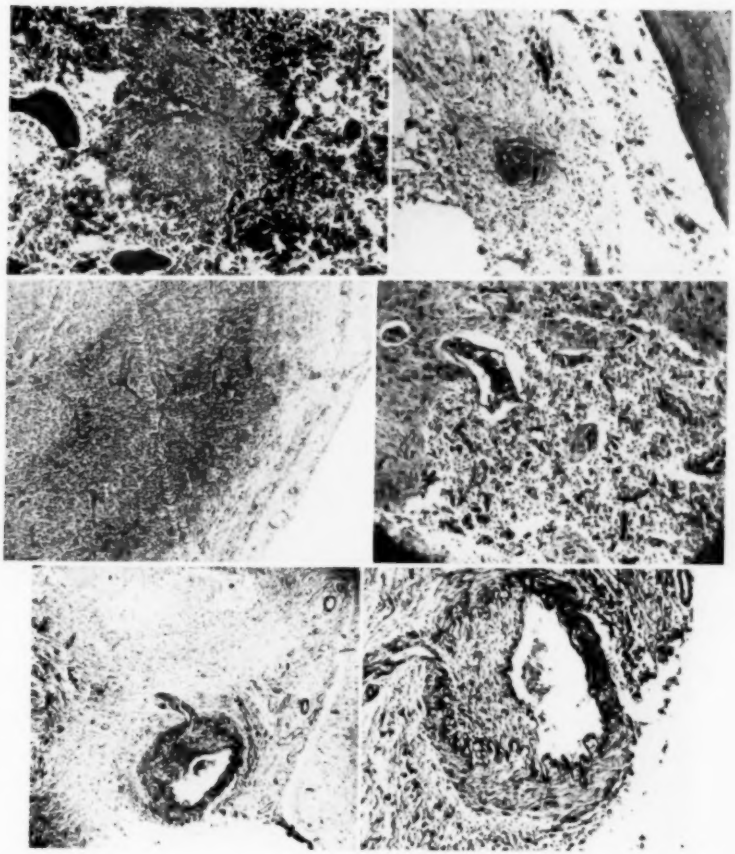
FIGS. 3–5

FIG. 3a. (Upper left) Case 1. Liver containing healed tubercle; complete fibrosis. (Streptomycin-treated)

FIG. 3b. (Upper right) Case 3. Liver with healed tubercle; complete fibrosis. (Streptomycin-treated)

FIG. 4. (Lower left) Case 1. Spleen containing healed tubercle; complete fibrosis. (Streptomycin-treated)

FIG. 5. (Lower right) Case 1. Kidney with healed tubercle; complete fibrosis. (Streptomycin-treated)



FIGS. 6–8b

FIG. 6a. (Upper left) Case 1. Bone marrow with healed tubercle; complete fibrosis. (Streptomycin-treated)

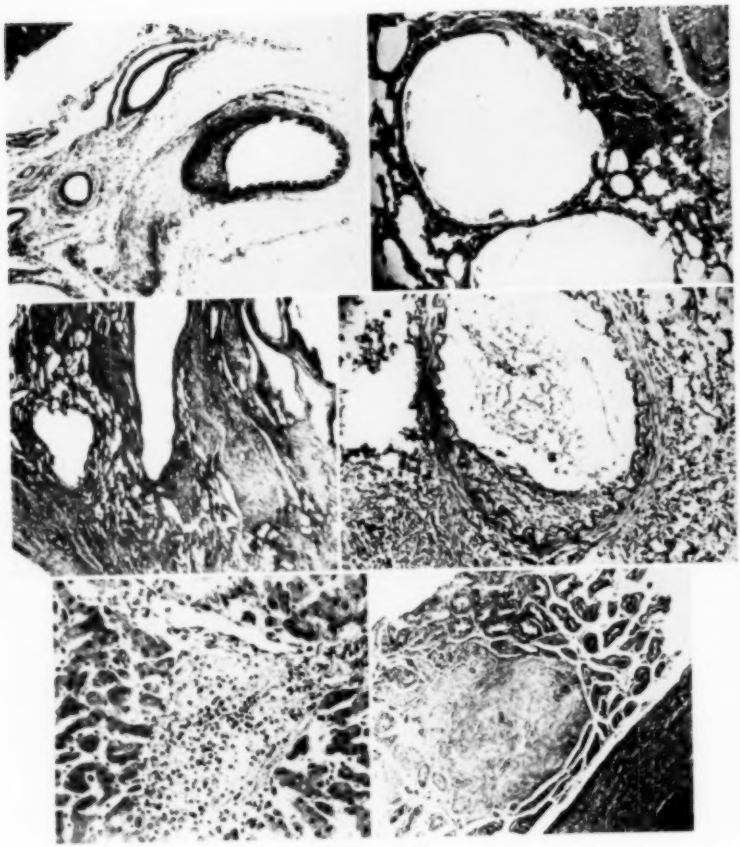
FIG. 6b (Upper right) Case 3. Healed tubercle in marrow of rib; complete fibrosis. (Streptomycin-treated)

FIG. 7a. (Center left) Case 1. Brain with marginal zone of a large caseous tuberculoma in right lower corner; fibrosis of overlying meninges (left). (Streptomycin-treated)

FIG. 7b. (Center right) Case 2. Focus of granulation tissue within cerebral parenchyma, contiguous with overlying fibrosed meninges. Probable healed tuberculoma. (Streptomycin-treated)

FIG. 8a. (Lower left) Case 2. Intimal hyperplasia in medium-sized artery, with meningeal fibrosis in immediate vicinity and caseation above. (Streptomycin-treated)

FIG. 8b. (Lower right) Case 2. Same vessel, higher power of magnification. (Streptomycin-treated)



FIGS. 8c–11b

FIG. 8c. (Upper left) Case 3. Intimal hyperplasia in medium-sized artery. Fibrotic, thickened meninges. (Streptomycin-treated)

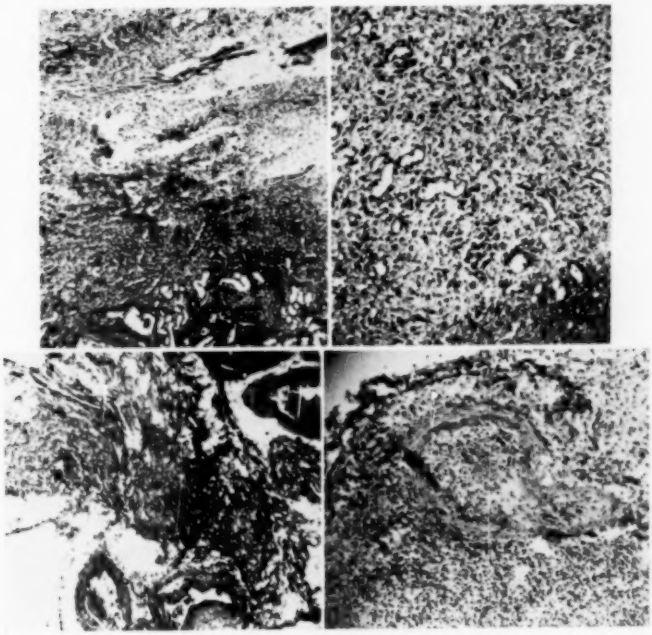
FIG. 9a. (Upper right) Case 1. Dilated bronchioli and emphysematous bullae in lung. (Streptomycin-treated)

FIG. 9b. (Center left) Case 3. Lung with marked fibrosis, dilated bronchioli. (Streptomycin-treated)

FIG. 10. (Center right) Intimal hyperplasia in medium-sized artery in meninges, showing acute exudative, caseous, and chronic fibrosing tuberculous meningitis. (No streptomycin therapy.)

FIG. 11a. (Lower left) Case 7. Liver containing healed tubercle. (Streptomycin-treated)

FIG. 11b. (Lower right) Case 6. Healing with scarring in testicle. (Streptomycin-treated)



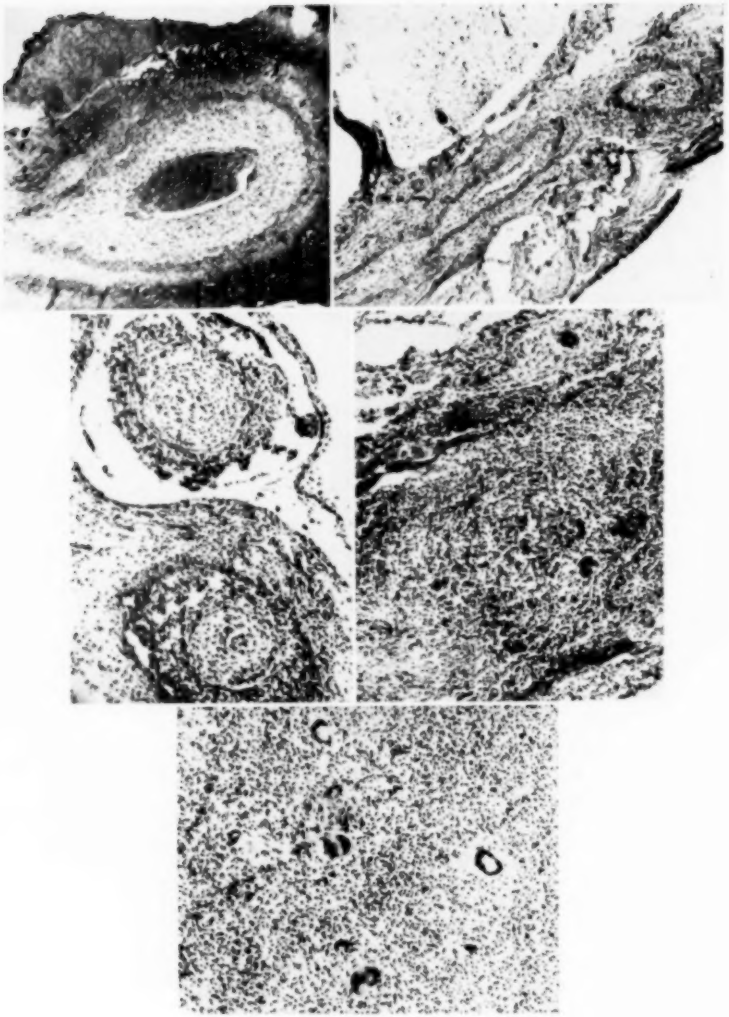
FIGS. 12–15

FIG. 12. (Upper left) Case 8. Region of renal pelvis showing fibrosis of papillae, probably representing a healed tuberculous pyelonephritis. (Streptomycin-treated)

FIG. 13. (Upper right) Case 6. Small focus of granulation tissue in brain, replacing either an area of softening or a small tuberculoma. (Streptomycin-treated)

FIG. 14. (Lower left) Case 6. Meningeal fibrosis. (Streptomycin-treated)

FIG. 15. (Lower right) Case 4. Small artery with lumen occluded by organized thrombus, lying in meninges showing fibrosis and round-cell infiltration (seen not infrequently in non-streptomycin-treated cases). (Streptomycin-treated)



FIGS. 16–18

FIG. 16a. (Upper left) Case 7. Medium-sized artery with intimal hyperplasia in meninges. (Streptomycin-treated)

FIG. 16b. (Upper right) Case 8. Thickened intima in medium-sized arteries; fibrosis of meninges. (Streptomycin-treated)

FIG. 16c. (Center left) Case 8. Small-caliber arteries in meninges, with intimal hyperplasia and marked reduction in size of lumen. (Streptomycin-treated)

FIG. 17. (Center right) Case 12. Extensive productive tuberculosis in the lung. (Streptomycin-treated)

FIG. 18. (Bottom) Case 13. Chronic tuberculous pneumonia. Alveoli replaced by fibrous tissue with a round-cell infiltration and an unusual number of large giant cells. (Streptomycin-treated)

PATHOLOGY OF PULMONARY TUBERCULOSIS[1,2]

As Modified by Streptomycin Therapy

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INTRODUCTION AND REVIEW OF LITERATURE

Numerous chemotherapeutic agents have been used in the treatment of tuberculosis (1). Streptomycin was added to the list as a bacteriostatic agent by the report in January 1944 by Schatz, Bugie, and Waksman (2) of their isolation of this new antibiotic. In November 1944, Schatz and Waksman (3) reported that streptomycin exerted a bacteriostatic effect on *M. tuberculosis*. Additional studies by Waksman and his associates (4, 5, 6) definitely established the ability of streptomycin to retard the growth of the tubercle bacillus. Waksman considers this antibiotic to be a repressive, inhibitory, bacteriostatic agent, not bacteriocidal or bacteriolytic. Its effectiveness against the tubercle bacillus is due to its interference with bacillary cell division and with the utilization of essential metabolites by the bacillus.

Feldman and Hinshaw had been engaged in the study of the effects of various chemotherapeutic agents on experimental tuberculosis in guinea pigs; the effectiveness of sulfapyridine was reported in 1939, of Promin in 1940–1945, and of Promizole in 1944. It was natural for them to engage in a similar study of the effects of streptomycin. They published a preliminary report in 1944 (7) including the modified picture of the tuberculous lesions in the experimental pigs, especially the striking healing of miliary lesions.

Following the favorable results of the animal experimentation, they treated human cases of tuberculosis with the antibiotic (8, 9, 10), and their reports were so favorable that one of the most comprehensive evaluations of the effects of a therapeutic agent which has ever been undertaken was started. Since the fall of 1946, the Streptomycin Committee of the Veterans Administration (11) (with 48 Veterans Administration, Army, and Navy hospitals cooperating) and the Streptomycin Tuberculosis Research Project of the American Trudeau Society (12) have engaged in a study of the effectiveness of this antibiotic. As a result, streptomycin has been found to be an exceptional agent in the treatment of miliary tuberculosis and tuberculous meningitis; a very effective antibiotic in exudative tuberculosis of the lungs, reversing the progressive trend in about 80 per cent of such cases; a distinct aid in furthering the closure of thin-walled cavities and producing "conversion" of the sputum, as well as a stimulant to the healing of laryngotracheobronchial tuberculosis.

The Trudeau Report (12) also emphasized that, since the course of chronic progressive pulmonary tuberculosis is modified by the occurrence of acute episodes, streptomycin had great potentialities in combating such episodes. The "drug-fastness" developing during prolonged streptomycin therapy has limited its effectiveness, since the failure of

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[2] Presented before the Medical Section, as part of the symposium on *Streptomycin and Tuberculosis*, at the 45th annual meeting of the National Tuberculosis Association, Detroit, Michigan, May 3, 1949.

cavities to close, the persistence of tubercle bacilli in the sputum, and the occurrence of relapses have been frequently associated with the appearance of streptomycin-resistant bacilli. This emphasized the necessity of using streptomycin with other accepted methods of treatment such as collapse therapy and surgical excision.

Steenken and Wolinsky (13) agreed with Feldman and Hinshaw (7) that streptomycin retarded and brought about a more marked healing than that produced by any other chemotherapeutic agent previously tried. They noted that after 40 days of streptomycin therapy the gross and microscopic pathology resembled a chronic type of tuberculosis, quite similar to the pathology seen in vaccinated guinea pigs. Yet after 125 days of therapy viable bacilli still persisted and, had the animals been permitted to live, they would have developed a progressive tuberculous disease.

Flory and his associates (14) reported on the modification of the lesions in 7 patients with a generalized miliary tuberculosis which complicated some visceral form of the disease. All 7 showed some response to streptomycin for three weeks to three months after therapy was begun. Two died of an exacerbation of the visceral disease, and 2 died of chronic tuberculous meningitis. Each evidenced healing in the miliary tubercles in the lungs, liver, and spleen, and often in other tissues.

These investigators described several modifications of the miliary tubercles. Focal fibrotic scars were seen in varying numbers in all 7 cases. Some scars were small, only three to five alveoli long by one to two wide; others were considerably larger. The scars consisted of a loose fibrous collagenous network in which a few fibrocytes, macrophages, and lymphocytes were scattered; capillary blood vessels were buried in the scars. There were no acid-fast bacilli. One case had complete healing of all tubercles; in the other 6, active tubercles were also present with the scars.

A second type of change consisted of the recurrence of an active, rapidly spreading soft tubercle with many acid-fast bacilli in what was a partially healed miliary tubercle.

In the third type of modification of the lesions described by these writers, large tubercles up to 3 mm. with central necrosis surrounded by incomplete collagenous fibrous rings were noted. Outside of this ring another zone of necrosis with many acid-fast bacilli were present.

Flory *et al.* also noted various exaggerations of the collagen which normally surrounds the caseous centers of the miliary tubercles. In 3 cases cavities were present which showed no streptomycin effect. In 2 cases a primary complex had become more circumscribed and denser. Enlarged lymph nodes had subsided under streptomycin, yet when examined at autopsy they were caseous and, if any healing had occurred, it was obscured by the caseation. They compared the healing of the miliary foci to the tubercles seen in 20 cases (of generalized miliary tuberculosis) before streptomycin therapy. In those cases which had received no streptomycin, no healing was seen that resembled the scars in the streptomycin-treated cases.

Baggenstoss, Feldman, and Hinshaw (15) reported 5 fatal cases of miliary tuberculosis which were autopsied. They observed convincing evidence of the regression and healing of the miliary tubercles in the lungs, liver, and spleen in all but one, and this case had only a few days of streptomycin. They described the healing as fibrosis, hyalinization, and absence of caseation in the miliary tubercles, yet in 3 of the 5 cases tubercle bacilli were still present.

Auerbach (16) reported the autopsy findings in 7 cases of miliary and chronic pulmonary tuberculosis, and concluded that streptomycin had a greater effect in the healing of tuberculosis which involved surfaces, e.g., laryngeal or tracheobronchial disease. He commented on the prompt clearing of perifocal infiltrations. He believed that in the

lungs the miliary tubercles, acino-nodose lesion, and alveolar caseous pneumonia foci healed with greater frequency than in untreated cases. He concluded that streptomycin did not prevent dissemination.

OBSERVATIONS

Material

At Fitzsimons General Hospital, since the fall of 1946 when streptomycin therapy was begun, 56 cases of pulmonary tuberculosis which had been treated with streptomycin have been autopsied. In the same period the surgical staff has removed 6 lungs (2 right and 4 left), 46 lobes, and 3 segments or wedges of lobes. The location of these lobes and segments were: 28 right upper, one right upper and middle, 4 right middle, 9 right lower, 7 left upper. The age range of the patients was eleven to forty-three years, with an average of twenty-six years. Ten per cent of the group were Negroes. The surgical specimens were from patients with chronic pulmonary tuberculosis who had focalized their disease under bed-rest therapy. Approximately two-thirds of the patients had received some form of collapse therapy in addition to streptomycin.

Such autopsy and surgical material represents the partial or complete failure of streptomycin therapy. As a consequence, the findings should be interpreted with a not too critical attitude toward the effectiveness of streptomycin, as there are too many living proofs of the efficacy of this antibiotic.

AUTOPSY FINDINGS

The following conclusions were reached as to the effect of streptomycin upon the tuberculous disease in the lungs of the 56 autopsied cases.

Miliary Tuberculosis, Pulmonary

In 13 patients with generalized miliary tuberculosis, 8 of whom died of a tuberculous meningitis, the streptomycin effect was definite and striking in the fresh submiliary and miliary tubercles in the lungs. In cases not treated with streptomycin the miliary tubercles and acino-nodose lesions proceeded from the epithelioid giant cell phase to caseation and necrosis, with surrounding small zones of perifocal reaction. In cases of "chronic miliary tuberculosis" not treated with streptomycin, the tubercles became encapsulated by fibrous walls with clearing of the perifocal reaction and the frequent appearance of emphysematous alveoli about these fibrocaseous miliary tubercles. Individual tubercles might even fibrose completely. Rich (21) has a microphotograph of a scarred tubercle, similar to those attributed to streptomycin; the caption beneath it is "healing in epituberculosis."

In the streptomycin-treated cases the progression of the individual tubercles had apparently been aborted and fibrous regression to complete scarring was substituted, not in occasional tubercles but in the majority of the lesions. In disseminated pulmonary miliary tuberculosis, the serial roentgenograms demonstrated either complete clearing of the miliary densities or incomplete clearing, leaving minute faint opacities. Corresponding to the roentgenographic inter-

pretation of complete clearing, the gross lung specimens had normal-appearing cut surfaces. Where the roentgenograms showed persistent faint densities, the cut surfaces were speckled with minute grayish dots, distributed in the pattern of the original miliary dissemination demonstrated on the film. Microscopic study of all the lungs showed fibrous scars at the sites of the grayish speckling seen grossly. These scars were irregular, oval, or stellate. Their size corresponded to that of a few to a dozen alveoli. The larger scars suggested by their arrangement that they had formed by merging of smaller lesions. Some represented the scarring of small groups of alveoli with the fibrous replacement in alveolar units. Other scars had no definite pattern in the laying down of the fibrous tissue and were considered to represent tubercles which arose in the interstitial tissue of the alveolar walls or interlobular septa. It was rare to find scars in the walls of the bronchi; this was attributed to the dual arterial supply of the lungs by the bronchial and pulmonary arteries.

The interstitial type of scar consisted of a loose fibroreticular meshwork, frequently carrying capillaries. Collagenation and hyalinization appeared with aging. In many instances the scars seemed to have been stretched by the elasticity of the parenchyma so that they appeared as nondescript fibrous thickenings amid air-containing alveoli which at times were emphysematous. Lymphocytes, occasionally plasma cells, and fibrocytes were scattered through these scars. Swollen, degenerated, vacuolated epithelioid cells persisted in the interstices of the loose fibrous meshwork in some cases. Occasional giant cells and residual lymphocytes of the original lymphocytic mantle were still evident in the less mature scars. No calcification was seen and none was expected, as streptomycin had presumably prevented caseation which is the precursor to calcification. Tubercle bacilli were absent from these scars. The various stages of the development of these scars from submiliary and miliary epithelioid tubercles could be followed by comparing sections from several cases, and occasionally could be seen in a single case where, after one shower of the tubercles had cleared, a fresh miliary spread occurred. In these relapsing cases it was apparent that, although streptomycin would control the miliary lesion of the spread, it had no permanent effect on the nodal, pulmonary, or extrapulmonary tuberculous focus which was the source of the spreads. In this series the shortest time required for the appearance of the streptomycin scarring of tubercles was 22 days; in another case it was present after 27 days of therapy.

In generalized miliary tuberculosis, streptomycin seemed more efficient in its suppression of pulmonary tubercles than in those in solid organs, as the spleen, liver, and kidneys. The reason for this is not definitely established; varying concentrations of streptomycin in the different organs and varying pH values do affect the efficacy of streptomycin.

The final impression was one of agreement with the generally accepted idea that streptomycin is effective against fresh miliary lesions. The scarred tubercles seen in streptomycin-treated cases were considered to represent the maximum effect of natural healing which was accelerated by streptomycin through its ability to inhibit the multiplication of the tubercle bacillus.

Chronic Pulmonary Tuberculosis

The following impressions were obtained as to the effect of streptomycin on chronic pulmonary fibrocaseous and fibrocavernous tuberculosis. The majority of the 30 patients in this group initially had shown some clinical and roentgenographic response to streptomycin followed by progression of the disease to a fatal end. As in all chronic pulmonary tuberculosis, it was difficult to distinguish between the natural processes of repair and the effects of the streptomycin therapy. Although in many the roentgenogram showed sharpening of the outlines and increased opacity of the nodular densities and sharper delineation of the cavities, microscopically it was impossible to arrive at any definite conclusion. In some, scattered miliary lesions had assumed the scarred appearance associated with streptomycin, but in the same areas there would be fresh caseous and necrotic miliary and acino-nodose lesions. Encapsulated areas of caseous pneumonia were not affected. Fresh caseous pneumonic areas showed no unusual features. The thick-walled cavities likewise revealed nothing that could be attributed to streptomycin. An occasional cavity showed more than the expected amount of granulation tissue in its lining, but intact submiliary tubercles of epithelioid giant cell type were often present in the wall of the cavity with none of the fibrotic increase expected from streptomycin therapy. The lack of evidence of streptomycin action could not always be attributed to drug-resistant organisms. Of the 30 cases, 19 had drug-resistant organisms, 6 had drug-sensitive bacilli, and 5 were not studied. In this group there were 3 cases of silico-tuberculosis. The pathologic findings in these cases were those of a progressive pulmonary tuberculosis; one had drug-sensitive organisms; in one the bacilli were drug-resistant; and the third could not be classified, as the organisms refused to grow sufficiently to permit proper determination of the drug sensitivity. No effect of the streptomycin therapy could be demonstrated in any of these 3 cases.

In this group of 30 cases, the clinical response to therapy was more evident than the roentgenographic or histopathologic evaluation. Twelve patients were considered to have had a good or moderate clinical response, 3 were classified as having good or moderate roentgenographic response, and only 2 could be considered to show a moderate histologic response to therapy.

One feature brought out by study of this group was the uselessness of continued streptomycin therapy after the tubercle bacilli had become resistant. One patient had 369 days of streptomycin therapy in three courses. The only response obtained was early in the first course when he improved clinically, but the roentgenograms never showed any improvement. Seven of these cases received over 180 days of streptomycin therapy and of these only 2 showed a moderate roentgenographic response, and only one a moderate histologic response.

The findings agree with those of other observers that streptomycin has limited use in chronic progressive fibrocaseous and fibrocavernous tuberculosis. Streptomycin can meet the challenge of tubercle bacilli when they are in the "open," so that perifocal reactions, fresh bronchogenic spreads of small extent, and satellite fresh tubercles can be halted in their progression and converted to a

regressive healing phase by streptomycin's bacteriostatic effect on the tubercle bacilli.

Nine patients with chronic pulmonary tuberculosis died from immediate cause other than tuberculosis; one after lobectomy, one from air embolism during a pneumothorax refill, 2 after thoracoplasty, 2 of cardiac disease, one of intestinal volvulus, one of uremia complicating severe renal tuberculosis, and one of carcinoma of the tongue. Two had drug-sensitive organisms, 5 had drug-resistant bacilli, and in 2 the cultures were not evaluated in terms of sensitivity. Six patients had good or moderate clinical response to therapy, 2 had moderate roentgenographic response, and 2 showed moderate histologic response evidenced by scarring of tubercles, increased fibrosis about caseo-nodose lesions, and increased granulation tissue in cavity lingings.

Four died of a disease other than tuberculosis: one of acute hepatitis, one of aplastic anemia, one of glomerular nephritis, and one of kala azar. It was thought that possibly natural tissue resistance would be so depressed in such cases that changes present in the tuberculous process might be more directly attributed to streptomycin. In none was there any indication of an unusual streptomycin effect.

Surgical specimens: From the examination of the 55 surgical specimens the following impressions were gained.

Peribronchial nodes: The peribronchial lymph nodes were all of reinfection type. They were received attached to the bronchi or about the hilum of the removed lobe or lung. Those that showed involvement were minimally involved. Scattered tubercles were seen along the sinuses and were predominantly of the sarcoid type, consisting of epithelioid cells with a few giant cells and rarely slight central caseation. Tubercle bacilli were rarely found in them and then only in small numbers. The consistency of the finding of these sarcoid tubercles, their small numbers, their scant fibrous capsules or the absence of capsules, and their minimal caseation suggested that only a few bacilli had reached the node or, having arrived in the node, they failed to multiply. This could well be a streptomycin effect.

Bronchi: Considering that two-thirds of the specimens had cavitation with viable bacilli in their linings, it was surprising how little tuberculosis of the bronchi was found; even in the bronchi which were directly draining the cavities. It was common to find groups of epithelioid cells with giant cells, groups of giant cells, and solitary giant cells scattered along the submucosa of the bronchi. They were infrequent in any one case. They suggested an unsuccessful attempt to form tubercles. The cells composing them seemed degenerating. Occasionally submiliary tubercles of epithelioid type were seen in the vicinity of the mucous glands of the larger bronchi; they showed no indication of caseating and were of indolent tuberculoid granuloma type. A search for tubercle blockage of draining bronchi as a cause for tension cavity was unsuccessful. In 2 cases with tension cavities diagnosed by roentgenogram, the incomplete blockage was due to sloughed pieces of the caseous lining of the cavity. These cavities showed more than the usual amount of granulation tissue in their linings which is probably

the reason for the sloughing. As streptomycin is acknowledged to have a marked effect on endobronchial tuberculosis, the minimal bronchial involvement noted in these surgical lungs could be considered a streptomycin effect.

Cavities: Various types of cavities were studied: chronic thin-walled, chronic thick-walled, small fresh ragged cavities in areas of encapsulated caseous pneumonia, and cavities resulting from fresh caseous excavations of small bronchi as a result of bronchogenic spread. Pagel (22) states that the spontaneous healing of a cavity is not common but does occur, especially when they are thin-walled and small. Streptomycin has acquired the reputation of accelerating the closure of thin-walled cavities. The thin-walled cavities were frequent in the surgical specimens. They tended to have little contents. The contents were soft, caseous, with many tubercle bacilli present. A thin layer of dirty, grayish, caseous slough coated their linings. Many polynuclear cells and occasionally some red blood cells were mixed with the caseous debris. Frequently the reddish color of the underlying granulation tissue showed through the overlying caseous necrotic slough. A layer of healthy vigorous granulation tissue devoid of epithelioid cell groups, of giant cells, and of tubercles formed the true lining. There was no caseation of its surface zone. In several the capillaries of the granulation layer were so numerous and dilated that they assumed a pseudo-angiomatous appearance. In such areas extravasation of blood was present in the stroma. Such engorged granulation tissue may explain the occasional reports that hemorrhage is more frequent in streptomycin-treated cases. Such healthy granulation tissue should be ideal for healing, for with spontaneous shrinkage or with collapse of the cavity by pneumothorax or other compression methods granulation surface would be apposed to granulation surface and fusion and healing should occur. Cavities in cases not treated with streptomycin often show granulation tissue in their linings but most often it is a sluggish tuberculous type with few and small capillaries, a compact stroma through which Langhan's giant cells, collections of epithelioid cells, and miliary tubercles are scattered. Often it gives the impression that, as it forms, its surface zone caseates.

The thick-walled caseous-filled cavities varied so greatly in the amount of caseation of their linings and in the thickness and the components of their fibrous walls that no decision could be made as to how much of the encapsulation was due to natural healing and how much to streptomycin.

The fresh small cavities seemed unaffected by streptomycin but, as stated above, in these surgical cases the prophylactic course of streptomycin had been in effect for only one week in most of the cases.

Parenchyma: The streptomycin type of scars of miliary tubercles were frequently seen, but in chronic pulmonary tuberculosis the miliary lesions play only a minor part in the disease process. They served a purpose in indicating that streptomycin had been at work. Fibrocaseous conglomerate tubercles were well encapsulated. Larger fibrocaseous nodules were not appreciably affected. The larger tuberculoma type of nodule was unaffected.

Concentration of streptomycin in lesion: Five patients with solitary tuberculomas varying from 2 to 4 cm. in diameter were treated with streptomycin not for the

purpose of curing the disease but to determine whether streptomycin could penetrate the fibrous, rather avascular walls of the lesions. The tuberculomas were removed and their caseous centers assayed for streptomycin. Four showed no streptomycin; one which had softening, early, slit-like cavitation with some granulation tissue lining the slit did show a trace. This would seem to indicate that streptomycin does not penetrate the solid fibrocaseous larger nodules.

In addition, the residuals of the perifocal infiltrations were found as described above.

In cases showing numerous giant cells, the foreign-body type far exceeded the Langhan's type in numbers. Asteroid bodies were more frequently seen in the foreign-body giant cells. This might be due to overzealous examination by the pathologist.

Visceral pleura: None of the lobes removed had tubercles or scars of tubercles in the pleura. Many had fibrous adhesions but no tubercles were found in them.

DISCUSSION

Among the autopsy cases, those with hematogenous miliary tuberculosis of the lungs were the most impressive as to the good effects of streptomycin. Such generalized hematogenous dissemination is the closest parallel in humans to a controlled animal experiment in which the intravenous inoculation route is used. In this type of tuberculosis the lesions are, relatively speaking, all of one age and one size for each seeding of the bacilli. So when organs involved by the miliary spread are examined, the recognition of active, healing and healed lesions is comparatively simple. On the other hand, this uniform picture is to be compared with the pathologic jig-saw puzzles presented by cases of subacute or chronic pulmonary tuberculosis. In such cases the lesions will include cavities, tuberculous and nonspecific pneumonic consolidations, nodose lesions of every type and size, perifocal reactions, various forms of bronchitis, solitary and conglomerate tubercles of every conceivable type, groups of epithelioid cells and giant cells not forming true tubercles, and even individual giant cells of Langhan's or foreign-body type. Multiply this picture by the varying ages of the lesions with the resulting varying phases of activity, regression or repair, add the modifications brought on by various types of compression therapy or by incidental pathologic changes such as atelectasis and bronchiectasis, and one may appreciate the difficulty the pathologist has in attempting to separate the natural variations of the disease from the modifications that streptomycin may have induced.

There are no specific pathologic changes peculiar to tuberculous lesions which had been treated with streptomycin. If streptomycin had some particular pathologic pattern; if it produced some specific cytological response, let us say a special type of giant cell or numbers of a particular cell like the eosinophil; or if it was associated with a characteristic type of tissue change such as amyloid, then its effects could be easily identified. Unfortunately, the healing changes induced by streptomycin are the same as the natural healing processes of the host. The clearing of perifocal exudative reactions, the closure of cavities, the

encapsulation of caseous foci, and the fibrotic healing of miliary lesions occur with or without streptomycin. In both situations the process is identical, as both are due to natural healing processes which are the standard for comparison. Nevertheless, streptomycin increases the frequency of the successful results and shortens the time required, as Muschenheim (19) stated.

In the material of the present study, no fresh massive exudative lesion of the perifocal type was found. Under streptomycin therapy such soft infiltrations clear rapidly. Amberson (20), in his study of the natural healing of such lesions, noted that practically complete resolution of most of the lesion could occur but that the older central portion persisted and was almost invariably necrotic by the time the total lesion was discovered. The fate of this necrotic focus depends on its size and on its establishment of a communication with a bronchus. It may soften, liquefy, and discharge its contents via a communicating bronchus, leaving a thin-walled cavity; such a cavity may close easily, especially if small. The necrotic focus may be encapsulated by a fibrous wall; as it ages, inspissation and calcification of its center occurs. In this phase it is a solid fibrocaseous nodule and may persist for life. Pagel (22) describes a similar exudative reaction under the terms "fugitive infiltrations" and "epituberculosis" especially in association with the primary parenchymal infection. He states that the subsequent course of such lesions may be (*1*) complete clearing, (*2*) thin-walled cavity, (*3*) encapsulated fibrocaseous nodule, (*4*) tuberculoid granulomata.

It is the anatomical structure of the lung with its bronchoalveolar units that compels practically every exudative inflammatory process, whether due to tubercle bacilli or to any other agent, to assume the microscopic or gross appearance of a lobular, segmental, or lobar pneumonia. Ultimately in such areas the native response of tissue to the presence of living or dead tubercle bacilli will be evidenced by the appearance of tubercles or tuberculoid granulomas.

As a result of streptomycin therapy, the resolution of the exudative infiltrations follows this same process except that it is more rapid. Serial roentgenograms of such perifocal infiltrations show the appearance of cavities, nodules, and scattered densities of varying "hardness" as the soft diffuse shadows clear.

Examination of lungs which had such infiltrations showed only the residuals. In some the only remaining evidence was fibrotic accentuation of interlobular trabeculae, thickening of the broncho vascular supporting meshwork, and scattered submiliary collections of lymphocytes. In some, tuberculoid granulomata, devoid of acid-fast bacilli, were scattered through areas of increased interstitial fibrosis with scattered alveolar lumina plugged with swollen, noncaseating, epithelioid cells. These granulomata are entirely different from the scarred miliary tubercles which were also present. In other cases, fibrocaseous nodules were present which were encapsulated areas of caseous pneumonia. In some of these fibrocaseous nodules, the elastica of the alveolar walls framed the alveolar lumina, filled and distended with ghosts of necrotic cells. Occasionally, small areas of fresh perifocal reaction were observed around foci of recent bronchogenic spread; no streptomycin effect was evident in these fresh areas. None was expected as the therapeutic streptomycin regimens had been stopped

at least one month previously, and in the surgical specimens the prophylactic course of streptomycin for coverage of the operation had lasted only one week before the lobectomy or pneumonectomy was done.

Thus it may be said that the effectiveness of streptomycin therapy is dependent on the type of lesion which is harboring the tubercle bacilli. In order for streptomycin to be effective, the bacilli must be sensitive, and must be accessible, to the antibiotic. The small nodular type of lesion is the most favorably affected. The acute exudative lesion is changed to a regressive phase by streptomycin and it prepares thin-walled cavities for closure.

In other words, streptomycin is not a cure. In pulmonary and miliary tuberculosis it suppresses acute and subacute exudations, and the effect of the drug is an excellent aid in collapse therapy and surgical excision.

SUMMARY

From the fall of 1946 to April 1949, 56 cases of pulmonary tuberculosis treated with streptomycin were autopsied; of these 13 were cases of generalized miliary tuberculosis.

In the same period 55 lungs, lobes, or segments (not including tuberculomas) were removed surgically from patients with pulmonary tuberculosis who had streptomycin therapy.

The cases of hematogenous miliary tuberculosis of the lungs were the most impressive as to the efficacy of streptomycin. A particular type of scar was seen replacing the miliary tubercle in the tissues of those patients who had responded clinically to streptomycin. The development of these scars could be followed. In relapse of the miliary disease, the failure of such scars to appear in the fresh spreads usually indicated that drug-resistant tubercle bacilli had become predominant. The shortest period of time required for the scars to develop was twenty-two days after the start of therapy.

Fresh massive exudative lesions were not represented in this series except by their residuals of cavities, fibrocaseous nodules, tuberculoid granulomata, nondescript fibrotic scarring, and focal collections of lymphocytes.

In cases of chronic fibrocaseous and fibrocavernous tuberculosis, the effects of streptomycin were indefinite.

The continuation of streptomycin therapy after the bacilli have become resistant to the drug *in vitro* had no further effect in restraining the natural progress of the disease.

The natural tissue healing was not affected by streptomycin except indirectly by reducing the bacillary population and thus accelerating the repair processes.

The sarcoid type of tubercle present in the lymph nodes of the surgical specimens suggested a streptomycin effect.

The lack of active endobronchial tuberculosis in the surgical specimens and the presence of an abortive type of tubercle in the bronchial walls might be considered to represent a streptomycin effect.

Streptomycin appeared to have a definite effect on thin-walled cavities in promoting a healthy granulation lining.

Thick-walled cavities and the larger fibrocaseous nodules, including tuberculomas, were unaffected by streptomycin.

Assay of the caseous centers of tuberculomas indicated that streptomycin did not penetrate such lesions.

In the streptomycin-treated cases the visceral pleura was free of tubercles.

SUMARIO

Anatomía Patológica de la Tuberculosis Pulmonar: Según la Modifica la Estreptomicinoterapia

Desde el otoño de 1946 hasta abril de 1949, hiciéronse autopsias en 56 casos de tuberculosis pulmonar tratados con estreptomicina, siendo 13 de ellos de granulia.

Durante el mismo período de tiempo extirpáronse quirúrgicamente 55 pulmones, lóbulos o segmentos (sin comprender tuberculomas) a tuberculosos pulmonares que habían recibido la estreptomicinoterapia. Los casos de tuberculosis miliar (granulia) hematógena de los pulmones fueron los más convincentes en cuanto a la eficacia de la estreptomicina. Observóse una cicatriz de forma peculiar que suplantaba al tubérculo miliar en los enfermos que respondían a la estreptomicina, pudiendo seguirse el desarrollo de dichas cicatrices. En las recidivas de la granulia, la inexistencia de esas cicatrices en las difusiones recientes indicaba por lo general que había aparecido resistencia. El período más breve de tiempo requerido para la aparición de las cicatrices fué de veintidós días.

En esta serie no estuvieron representadas las lesiones exudativas masivas recientes, salvo por sus residuos de cavernas, nódulos fibrocaseosos, granulomas tuberculoideos, cicatrización fibrosa confusa y depósitos focales de linfocitos.

En los casos de tuberculosis fibrocaseosa y fibrocavernosa crónica, los efectos de la estreptomicina fueron imprecisos.

La continuación de la estreptomicinoterapia después de volverse resistentes los bacilos no ejerció efecto ulterior en lo tocante a contener la evolución natural de la enfermedad.

La cicatrización histológica normal no se ve afectada por la estreptomicina, excepto indirectamente, por reducir la población bacilar y acelerar así los procesos de regeneración.

La forma sarcoidea del tubérculo presente en los ganglios linfáticos de los ejemplares quirúrgicos indicaba efecto de la estreptomicina.

La ausencia de tuberculosis endobronquial activa en los ejemplares quirúrgicos y la presencia de una forma abortiva de tubérculo en las paredes bronquiales podría ser efecto de la estreptomicina.

La estreptomicina pareció ejercer efecto bien definido sobre las cavernas de paredes delgadas por fomentar un recubrimiento sano de granulación.

Las cavernas de paredes gruesas y los nódulos fibrocaseosos mayores, incluso tuberculomas, no fueron afectadas por la estreptomicina.

El estudio de los centros caseosos de los tuberculomas indicó que la estreptomicina no penetraba en esas lesiones.

En los casos tratados con estreptomicina la pleura visceral estaba exenta de tubérculos.

CONCLUSIONES

La efectividad de la estreptomicinoterapia depende de la forma de lesión que albergue los bacilos tuberculosos. Para que la estreptomicina sea eficaz, los bacilos tienen que ser sensibles a la misma y accesibles al antibiótico. La pequeña lesión de forma nodular es la afectada más favorablemente. La lesión exudativa aguda pasa a una fase regresiva por virtud del efecto de la estreptomicina. Esta prepara para el cierre las cavernas de paredes delgadas.

En otras palabras, la estreptomicina no es una cura. En la tuberculosis pulmonar, suprime los exudados agudos y subagudos y la granulia, y constituye un excelente auxiliar en la colapsoterapia y la excisión cruenta.

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EFFECT OF INTRAVENOUS TUBERCULIN INJECTIONS ON SUBSEQUENT TUBERCULIN SKIN REACTIONS IN HYPERSENSITIVE RABBITS[1,2]

CURTIS SANDAGE[3] AND JORGEN M. BIRKELAND

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INTRODUCTION

One of the most familiar distinguishing characteristics of tuberculin-type hypersensitivity is the delayed and prolonged nature of the local tuberculin skin reaction. While Rich (1) has suggested that "the gradual and progressive character of the inflammation, rather than a latent period of delay in the occurrence of inflammation" should be emphasized, the fact remains that the height of a local tuberculin reaction is not reached until many hours after the intradermal injection of tuberculin.

In the past it was frequently suggested that a causal relationship exists between the focal tuberculin reaction on the one hand and the local and systemic reactions on the other. This proposal is based largely on the hypothesis that the focal reaction results in the liberation of a "toxic substance" which is involved in the subsequent course of events. Rich (1) has reviewed the evidence for and against this theory and concludes that the data in support of it are inadequate. The fact is that a satisfactory explanation for the delayed tuberculin reaction is still lacking.

The work reported here was done in the course of re-examining, in the light of more recent developments, certain theories of the mechanism of the tuberculin reaction. As a working hypothesis, it was assumed that the tuberculin skin reaction is delayed because it must be preceded by some kind of systemic reaction.

EXPERIMENTAL

White male rabbits, weighing 2.5 to 3.0 Kg. each, were injected subcutaneously with living cultures of various strains of *M. tuberculosis*. The animals were used six to eight weeks later, at a time when skin sensitivity to tuberculin was fully developed.

Some of the hypersensitive animals were injected intravenously with various quantities of tuberculin (PPD). After intervals of time ranging from 8 to 24 hours these "treated" animals were skin-tested with PPD. The quantity injected intradermally was usually 0.2 mg. in a volume of 0.2 ml. Control groups, skin-tested in an identical manner, included infected animals which had not been given tuberculin intravenously and uninfected normal rabbits which had been injected intravenously with tuberculin.

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[2] This work was supported by a grant from The Ohio Tuberculosis and Health Association.

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Following intradermal injection of PPD, the skin reactions were observed at hourly intervals for the first 15 hours and at 3- to 4-hour intervals for the next 12 hours. The area and intensity of erythema, height of induration, and degree of central blanching was recorded.

RESULTS

The results obtained in a typical experiment are shown in table 1. As may be seen, the time required for the tuberculin reaction to reach its height was reduced by about two-thirds in those animals which had been injected intravenously with tuberculin 12 hours prior to skin-testing. It is significant that in animals with such accelerated skin reactions, the erythema was restricted to a much smaller area and the reactions were considerably more intense. Petechial hemor-

TABLE 1

Skin Response of Hypersensitive Rabbits to 0.2 mg. (0.2 ml.) of PPD 12 Hours after Intravenous Injection of Tuberculin

INFECTIVE AGENT	ANIMAL NUMBER	INTRAVENOUS TREATMENT	TIME FOR MAXIMUM SKIN REACTION	AREA OF ERYTHEMA IN MM.²*
			hours	
BCG	5	0.10 mg. PPD	7 to 9	60
	6	0.02 mg. PPD	8 to 10	78†
	7	none	24 to 30	610
Ravenel	9	0.10 mg. PPD	10 to 12	110†
	10	0.02 mg. PPD	8 to 10	92
	14	none	30 to 36	685
None	28	0.1 mg. PPD	—	No reaction
	29	0.5 mg. PPD	—	

* Area at height of reaction.

† Petechial hemorrhage present.

rhage was commonly observed, particularly at the periphery of the zone of erythema.

The 6 hypersensitive rabbits shown in table 1 and suitable controls were tested in an identical manner one month later. The results were essentially the same.

A total of 36 tuberculin-sensitive rabbits have been used for similar experiments. The results may be summarized as follows. Under the conditions of these experiments, in rabbits with well-developed tuberculin skin sensitivity, the maximum skin response was attained approximately 30 hours after intradermal injection of PPD. In strong reactions, induration and some central blanching was observed, but the reactions did not progress to necrosis. When such animals were given tuberculin intravenously some hours prior to skin-testing, the skin reaction reached a maximum in one-fourth to one-half the usual time. Such reactions were more intense but smaller in area. The extent of deviation from the normal response appeared to depend upon three factors: (*1*) the degree of sensitivity of the animal, (*2*) the quantity of tuberculin injected, and (*3*) the length of time allowed between the two tuberculin injections.

DISCUSSION

These results suggest that the delay in cutaneous response to tuberculin is due in part to the time required for absorption of tuberculin from the site of injection. Furthermore, it appears that the skin response is secondary to a "primary reaction," as yet unidentified, which can be elicited by injection of tuberculin intravenously. If this "primary reaction" results in the release into the circulation of a substance with "antibody-like" specificity for tuberculin, its presence in the blood, following the intravenous injection of tuberculin, could account for the accelerated skin response to tuberculin. It might also account for both the decrease in size and the increase in intensity of such reactions. Presumably, a given quantity of residual tuberculin would result in more intensive tissue damage if reactive "antibody" reached it before it had diffused over a larger area of tissue.

Chase (2) and others (3-5) have found that the delayed tuberculin reaction can be produced in normal animals following the injection of leukocytes from a tuberculin-sensitive donor. This well-established phenomenon is proof that the focal reaction, in the usual sense of the term, is not required per se for the delayed tuberculin skin reaction. Nevertheless, it seems highly significant that in animals passively sensitized with leukocytes, the skin reaction to tuberculin is still delayed.

Favour (6) has shown that leukocytes of tuberculin-sensitive animals are lysed *in vitro* by tuberculin. It is possible that a similar *in vivo* reaction may be the postulated "primary reaction" which precedes the delayed skin response, whether in the actively or passively sensitized animal. Available evidence seems to indicate that lysis of the lymphocyte may play a major role in the development of the tuberculin reaction. Stavitsky (4) succeeded in passively transferring tuberculin sensitivity with a cell suspension consisting of 95 per cent lymphocytes. Favour (6) reported that *in vitro* lysis of lymphocytes by tuberculin precedes lysis of other types of white cells and that, if lympholysis is prevented, no damage to other cells occurs. If a liberation product of lympholysis with an "antibody-like" specificity for tuberculin were required for the development of the tuberculin skin reaction, it would account for the accelerated reactions which were obtained in the experiments reported here.

It should be emphasized that the term "antibody" is used entirely for want of a better term. As Chase (2) has pointed out, the factor in leukocyte suspensions which is apparently responsible for passive transfer is extremely labile. In unpublished experiments in this laboratory, it has been found that a substance which appears in the blood during tuberculin shock is also labile. This substance apparently possesses the capacity to confer tuberculin sensitivity on normal animals and to lyse red blood cells.

SUMMARY

In tuberculin-hypersensitive rabbits, intravenous injection of PPD some hours before skin-testing resulted in an accelerated cutaneous reaction. Such reactions were smaller in area and more intense than were those in control

animals. These results and data reported by others are discussed in an effort to explain the mechanism of the delayed tuberculin reaction.

SUMARIO

Efecto de las Inyecciones Intravenosas de Tuberculina sobre las Subsiguientes Cutirreacciones a la Tuberculina en Conejos Hipersensibles

En conejos hipersensibles a la tuberculina, la inyección endovenosa de PPD algunas horas antes de la cutirreacción produjo una reacción cutánea acelerada. Esas reacciones fueron más pequeñas en extensión y más intensas que las de los animales testigos. Tratando de explicar el mecanismo de la tuberculinorreacción demorada, discútense dichos resultados y los datos presentados por otros autores.

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THE DISTRIBUTION OF IRON IN TUBERCULOUS GRANULATION TISSUE[1, 2]

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INTRODUCTION

In a number of papers, Menkin (1–6) has reported on the accumulation of iron in tuberculous lesions of rabbits and on the favorable effect of intravenous injections of iron on the course of experimental tuberculosis. In this laboratory, the findings of Menkin could not be confirmed (7); it was found that iron is not deposited in tubercles and does not exert any noticeable therapeutic effect.

EXPERIMENTAL

As a sideline to the experiments on the effect of parenterally administered iron, the histologic distribution of iron in the organs of various species (man, mouse, rat, rabbit, guinea pig, and cat) infected with tuberculosis was also studied. The only species in which iron was administered intravenously was the rabbit; in the others, the iron studied was endogenous.

Human tissues were not surgical biopsy specimens, but were obtained at autopsies. The animals were infected by the intravenous or subcutaneous injection of a suspension of virulent organisms. Mice, rats, and cats were given 0.1, 0.3, and 1.0 mg., respectively, of an H37 strain intravenously; guinea pigs, 1.0 or 2.0 mg. of the same strain subcutaneously; rabbits, 1.0 mg. of a bovine strain (Ravenel) subcutaneously. Six mice, 6 rats, 4 cats, 20 guinea pigs, and 20 rabbits were used. The animals were killed or allowed to die after an interval of three to six weeks. The organs were fixed in formalin-alcohol (1:4); sections were stained for Prussian blue by an acid-ferrocyanide method (8). Neutral red was used as a counterstain.

RESULTS

All animals developed a more or less extensive infection, with lesions showing the typical microscopic structure of tuberculosis (varying somewhat with the different species) and containing large numbers of acid-fast organisms.

In all species except the rat, both the specific granulation tissue and necroses were conspicuously free from iron, even when much iron was seen in other surrounding tissue elements such as the macrophages of the spleen or the Kupffer-cells of the liver. In some of the iron-treated rabbits, traces of iron were observed in tuberculous granulation tissue, but the amount was much inferior to that scattered in the perifocal connective tissue. However, in the rat the situation was completely different. All tubercles, regardless of location, were stained deep blue; both epithelioid and giant cells were densely studded with granules of Prussian blue. Necroses were not seen in this species. The storage of iron in tu-

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berculous granulation tissue was so selective that the smallest tubercles stood out sharply in a deep blue shade.

The role of iron storage in the natural resistance of the rat to tuberculosis is

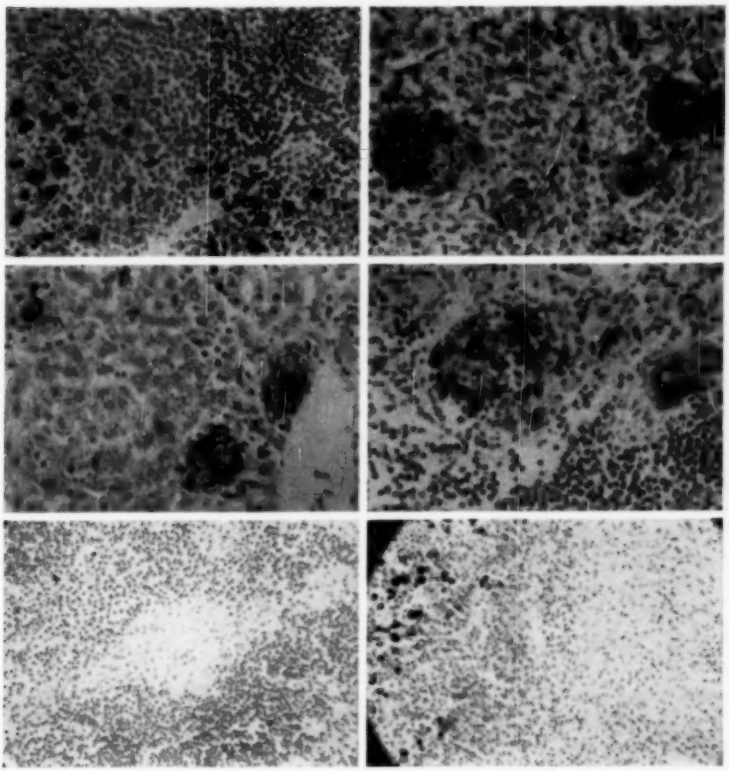

FIG. 1. (Upper left) Spleen of the normal rat. Iron present in macrophages.

FIG. 2. (Upper right) Tubercles in the spleen of the rat.

FIG. 3. (Center left) Tubercles in the liver of the rat.

FIG. 4. (Center right) A tubercle in a lymph node of the rat.

FIG. 5. (Lower left) Tubercles in the spleen of the mouse. Neither the normal tissue nor the tubercles show any reaction for iron.

FIG. 6. (Lower right) A large tubercle in the spleen of the guinea pig. Reaction for iron in perifocal macrophages only; lesion itself free from iron.

hard to evaluate, the more so as the mouse, practically as resistant as the rat, does not show it. Further experiments with a number of other species may help to decide whether there is any connection between iron storage and resistance to tuberculosis.

SUMMARY

Tuberculous granulation tissue of the rat stores ferric iron; similar lesions in other species are practically free from this element.

SUMARIO

La granulación tuberculosa de la rata almacena hierro férrico. Lesiones similares en otras especies estan casi por entero desprovistas de este elemento.

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METHODS FOR ISOLATING TUBERCLE BACILLI[1, 2, 3, 4]

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INTRODUCTION

During the past few years increasing importance has been attached to the cultivation of tubercle bacilli from pathogenic materials on synthetic media. The mycobacterium is notoriously difficult to grow from small concentrations. This is further aggravated by the appearance of contaminating organisms. For many years private investigators, as well as the committees of the American Trudeau Society, have conducted a diligent search for a medium which would be as sensitive as animal inoculations without the attendant considerable lapse of time for diagnosis. Many media have been developed which in the hands of trained technicians, especially those who devoted the major portion of their time to tuberculosis work, are as sensitive as guinea pig inoculations and in many cases more so.

The writers, in the course of their work, have become acquainted with the various media developed by Doctors Dubos, Middlebrook, and Pierce of the Rockefeller Institute for Medical Research. At first the liquid Tween-albumin medium was used for determination of streptomycin sensitivity. Since this medium required primary growth on a solid medium before the various concentrations of streptomycin could be inoculated, the whole process became quite time-consuming. It was felt that the procedure could be accelerated if a solid medium could be used, both for sensitivity tests and controls, upon which the pathogenic material could be planted directly (on streptomycin-containing media). Pyle (1) and Youmans (2), as well as Karlson (3), were guided by the same idea and used Herrold's medium (4) for the same purpose. After considerable experimentation, Dubos albumin agar medium (5) with the addition of 100,000 units of sodium penicillin per liter was used for sensitivity tests (6). The absence of Tween and serum overcame the objections of Fisher and Youmans (7, 8) pertaining to false reading of streptomycin resistance in the presence of Tween.

After considerable experience had been gained (more than 1,000 sensitivity tests and more than 1,500 diagnostic plates) in the use of the albumin agar medium and after many parallel tests had been run, it was decided to use Dubos medium exclusively for diagnostic as well as research purposes in the Arroyo Del Valle Sanatorium at Livermore, at the Alum Rock Sanatorium at San Jose,

[1] From the Arroyo Del Valle Sanatorium, Alameda County, California.

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[3] Presented in part at the annual meeting of the California Trudeau Society, April 4, 1949.

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and at Arequipa Sanatorium in Marin County, California. The specimens for study were obtained from our patients, from Highland and Fairmont Hospitals of Alameda County, as well as through the aid of private physicians. The experiments which led to the adoption of this policy are presented below.

TECHNIQUE

Specimens of sputum, tracheal or gastric lavages, et cetera, are concentrated in the usual manner with 3 per cent sodium hydroxide, shaken for one hour and neutralized with 3 per cent sulfuric acid immediately upon removing from the shaking machine. After centrifuging and obtaining a sediment, it was found that a more homogenous and faster growth is obtained when the sediment is suspended in liquid Dubos (albumin-oleic acid) or buffered distilled water and shaken for a few minutes. Saline was not used on account of the possible effect upon the viability of the bacteria. One-tenth cc. of suspension is then poured on the medium in 60 mm. Petri dishes, and the material is then evenly distributed with a platinum loop. The plates are sealed with cellophane tape and placed in cans and the latter are stored in the incubator for one week when the first readings are made. Material containing very small concentrations of tubercle bacilli are not exposed to light for 9 to 10 days. The small Petri dishes mentioned above lend themselves very well to this purpose, particularly in view of the fact that they are economical (only 10 to 12 cc. of medium are required) and can be examined under low power on any microscope without disturbing the growth of bacteria. Since this is a translucent medium, growth can be microscopically detected before there is any macroscopic evidence. In most cases, the appearance of the colonies is so typical that they cannot be mistaken for anything else. Good illustrations of the colonial morphology may be seen in the article by Middlebrook, Dubos, and Pierce (11). If there is any doubt at all, smears should be made. Culture of the usual sputum specimen containing tubercle bacilli (the type used for sensitivity tests) yields visible growth in 7 to 12 days. Sputum specimens less rich in bacilli, gastric lavages, or tracheal washes usually require from 10 to 22 days before the appearance of growth. It is only very rarely that a positive culture is first observed after more than 24 days of incubation. All plates which are negative after 30 days of incubation are discarded.

After several tests had been undertaken with stock cultures which had been diluted to such minute amounts as 10^{-5} and 10^{-6} mg. per cc. and the Dubos medium had been found superior to the others, an elaborate test with clinical specimens was set up.

EXPERIMENTAL OBSERVATIONS

It was believed that it would be preferable to conduct the experiments with material obtained directly from infected patients rather than with various dilutions of suspensions of tubercle bacilli. Such a test would approximate clinical laboratory conditions to a much greater extent. The Veterans Administration hospitals had been urged to use a modification of Herrold's medium similar to the one described by Karlson *et al.* (4). The ATS media have been recommended by the Committee on Laboratory Procedure of the American Trudeau Society (10). Petrick's medium had been used for several years at the laboratories of Alameda County and the staff was quite familiar with its use. Thus, it was felt that it would be significant if these media were compared with Dubos albumin agar medium. The experiments were set up according to R. A. Fisher's method of the Latin Square and the same number of replications were used as there were variants. Thus, a statistical analysis of the results was made more easy of accomplishment. As it turned out, the results were so one-sided that such an analysis was unnecessary.

It will be seen (table 1) that Dubos albumin agar showed growth in all but one instance. This superiority was particularly apparent in tests concerned with the smaller concentrations, where the other media produced growth on occasional plates while Dubos medium was positive in all but one case. It appears that Herrold's medium, without penicillin, is probably not suitable for use for primary isolation (for diagnostic purposes) while Herrold's medium, with penicillin, gives a pretty good account of itself. The ATS medium, at least in our hands, was not as satisfactory in this particular test. It should be stated, however, that during the past twelve months the ATS medium was used routinely along with

TABLE 1

	Sputum B*					Sputum D†					Sputum M‡					Control	
	Replications					Replications					Replications						
Type of culture medium	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2
ATS medium[b]	9[a]	9	9	NG	22	NG	NG	31	NG	NG	27	17	NG	NG	NG	NG	NG
Petrick's	22	13	13	13	NG	NG	NG	NG	NG	NG	NG	20	34	22	NG	NG	NG
Herrold's[d]	9,[c]	9,	9,	15,	9,	NG	NG	NG	NG	NG	C	C	C	C	C	NG	NG
Herrold's with penicillin[e]	9[a]	9	9	9	9	NG	NG	32	NG	45	39	22	45	NG	NG	NG	NG
Dubos albumin agar[e, f]	9[a]	9	9	9	9	16	C	17	22	14	17	17	17	17	17	NG	NG
Guinea pig	Positive (died 45 days)					Positive (was killed)					Positive (died 60 days)						

* Sputum B was a Gaffky 5 sputum.

† Sputum D was from a patient who was intermittently positive to culture but always negative to concentrated smear (microscopically).

‡ From a patient negative to smear (microscopically) but usually positive to gastric lavage on Dubos culture.

In all cases a suspension of the sediment was made in liquid Dubos (oleic acid). The material was shaken for five minutes and equal amounts were planted in each variant and replication.

[a] The numbers refer to the number of days elapsed until growth could be seen. In the case of "9," this means 9 days *or less*, since the cans were not opened until 9 days had elapsed. "NG" means no growth.

[b] This is the standard medium recommended by the American Trudeau Society Committee on Evaluation of Laboratory Procedures (10).

[c] Contamination present—diagnosis of *M. tuberculosis* still possible.

C Overwhelming contamination.

[d] Medium used by Pyle, Karlson, *et al.*, as well as the Veterans Administration hospitals, Proc. Staff Mayo Clinic, Vol. 23, Number 18, also Vol. 22, 465.

[e] Sodium penicillin was added at the rate of 100 units per cc.

[f] It was found to be advisable to use .05 Gm. ferric ammonium citrate, 2.0 Gm. of glucose, 1.0 Gm. of asparagin, 1.0 Gm. of casein. It is quite important to grow these bacteria at 6.5 pH. A MacBeth pH meter has been found to be very suitable for this purpose.

Dubos albumin agar in culturing specimens from every patient who received streptomycin. Under these conditions, the ATS medium yielded growth of *M. tuberculosis* in 88 per cent of all instances when Dubos agar was positive, while the former medium was positive in only 7 instances (out of 512) where Dubos agar yielded no growth.

Synthetic Culture Media vs. Guinea Pigs

Specimens from a series of 421 consecutive cases were studied at the VA Hospital at Livermore. An aliquot of each specimen was inoculated into a guinea pig. The results in the first 213 specimens were as follows:

Total cultures	213
Total positives for *M. tuberculosis* on culture	62
Total positives for *M. tuberculosis* to guinea pig	51
Negative on animal inoculation, positive on culture	11

(The culture medium was an inspissated egg yolk-potato medium similar to the ATS medium).

There were no positives on guinea pig inoculation which were negative on culture.

The next 208 specimens yielded the following results:

Total cultures	208
Total positives on culture	90
Total positives on guinea pig inoculation	58
Total positives on Dubos[1]	90
Total positives on egg yolk-potato	82
Negative on guinea pig inoculation, positive on culture	22

At Arroyo Del Valle and the other sanatoriums mentioned, another 169 cultures were made. In this case the guinea pig inoculations were compared with the results obtained on the albumin agar medium.

Total cultures	159
Total positives	117
Positive on animal inoculation	105
Negative on animal inoculation but positive on culture	12

Combining these data it is found that there was a total of 580 specimens from the same number of patients.

Positive on culture	269
Positive on guinea pig inoculation	224

DISCUSSION

In experiments of this type the familiarity of the workers with the various media plays a very important part in assaying the results. It is probable that results as striking as these are a consequence, not only of the intrinsic merit of the medium itself, but also reflect the fact that the investigators have become very well acquainted with the use of the medium in the course of concomitant research work. Since the writers did not have a similar acquaintance with the other media, except Petrick's, the results are weighed in favor of Dubos. In the tests where the Dubos media were compared with guinea pigs, a special study was set up and liquid Dubos containing Tween 80 was compared with animal inoculation.

The cultures recorded as positive were all examined by stained smear before final diagnosis. In all cases where the guinea pig was negative, the growth from culture was inoculated into pigs for confirmation of pathogenicity. Forty-two died of tuberculosis and in the other 3 the skin reaction was positive to 5.0 mg. of Old Tuberculin. There were another 6 cases in which specimens were positive to culture and negative on guinea pig inoculation, but which upon guinea pig inoculation of the isolated culture proved to be nonpathogenic. These 6 cases were considered positive on egg yolk-potato medium. No such experience oc-

[1] The medium used was the liquid Tween medium. Strands were smeared for diagnosis.

curred with the albumin agar cultures, for nonpathogenic organisms either did not grow (probably as a result of the penicillin) or their morphology was sufficiently distinctive so that they were not diagnosed as *M. tuberculosis*.

It is admitted that the tests with liquid Dubos have no practical application since the smearing of sediments from growth in (usually) contaminated liquid media is a laborious and tiresome process and not suitable for the average laboratory. It was done only to test the relative sensitivity. The comparison of *solid* Dubos with guinea pigs did not produce as striking results but proved that the medium is at least equal to animal inoculation in sensitivity. Whatever the modifying conditions, it has been established that a synthetic medium of satisfactory stability has been developed which is suitable for routine diagnostic work in tuberculosis laboratories.

The use of guinea pigs in general hospitals or general laboratories is not being discouraged. Unless considerable experience with culturing tubercle bacilli has been obtained, animal inoculation is still the safest method of diagnosis.

SUMMARY

Four media: ATS, Petrick's, Herrold's with penicillin, and Herrold's without penicillin have been compared with Dubos albumin agar (oleic acid) medium. It appears that the latter is superior to the above media for purposes of diagnostic culture of tubercle bacilli. Dubos (liquid) medium as well as Dubos (solid) medium have been compared with guinea pigs. It appears that Dubos (solid and liquid) media are somewhat superior to guinea pig inoculations with very low concentration of tubercle bacilli.

SUMARIO

Técnicas para el Aislamiento de los Bacilos Tuberculosos

Cuatro medios: el A. T. S., el de Petrick, el de Herrold con penicilina y el de Herrold sin penicilina, fueron comparados con el medio de Dubos, de agar-albúmina (ácido oleico). El último parece ser superior a los otros para fines de cultivo para diagnóstico de los bacilos tuberculosos. Los medios, tanto líquido cuanto sólido, de Dubos fueron comparados con la inoculación en el cobayo, pareciendo los primeros ser superiores cuando es muy baja la concentración de bacilos tuberculosos.

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A DIRECT METHOD FOR THE DETERMINATION OF THE SENSITIVITY OF TUBERCLE BACILLI TO STREPTOMYCIN[1, 2]

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INTRODUCTION

The determination of the sensitivity to streptomycin of tubercle bacilli isolated from patients under treatment with the drug continues to be an important part of the disease management. A variety of methods have been proposed using pure cultures of tubercle bacilli. Recently these have been reviewed by Williston and Youmans (1) who recommended the use of solid rather than liquid media, as the former furnished an opportunity to determine roughly the relative numbers of streptomycin-sensitive and -resistant bacilli in a culture. Karlson and Needham (2) have also published a method in which Herrold's egg yolk agar is recommended.

It has been suggested also that the employment of a solid culture medium might permit the determination of sensitivity at the time of primary isolation of the bacilli by direct inoculation of concentrates of sputum or other pathologic material on to the streptomycin-containing medium. If feasible, this method should furnish a more exact evaluation of streptomycin resistance than using transplanted pure cultures because nearly the total bacterial population of each patient's sputum is thus represented. Furthermore, the time required is no longer than that required for primary isolation of the tubercle bacilli.

The success of such a method involves the solution of many technical difficulties. Most of these problems have been effectively surmounted in the streptomycin laboratories of the Chicago Municipal Tuberculosis Sanatorium during the last two years. At this time the methods are being presented in detail, together with an analysis of the results obtained in performing streptomycin sensitivity tests on 2,009 sputum samples.

METHODS

The performance of direct streptomycin sensitivity tests involves inoculation of portions of well homogenized sputum concentrates on to media containing known concentrations of streptomycin and on similar media containing no streptomycin. These cultures are incubated at 37.5°C. and examined at appro-

[1] From the City of Chicago Municipal Tuberculosis Sanatorium Research Laboratories and the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

[2] This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service.

priate intervals. A record is made of the degree of sensitivity to streptomycin of the bacilli.

During the fourteen months that sensitivity tests have been performed directly from sputum concentrates, a variety of media and methods have been tried. Rather than engender confusion by describing media and methods no longer in use, only those found to be most suitable are presented in detail.

A. Nutrient Agar Egg Yolk Medium

Ingredients:

1. Difco nutrient dehydrated agar medium (B[1]) ..... 23 Gm.
2. Distilled water ..... 750 ml.
3. Glycerol (reagent grade) ..... 10.0 ml.
4. Malachite green (2 per cent aqueous solution)[2] ..... 10.0 ml.

The nutrient agar should be thoroughly dissolved in the distilled water by heating and shaking. The glycerol and Malachite green are added and the medium is dispensed in 75- or 150-ml. amounts in Erlenmeyer flasks which are then plugged with cotton and sterilized in the autoclave for twenty minutes at 20 pounds pressure. An equal quantity of medium is prepared for each concentration of streptomycin (1.0, 10, and 100 γ) and for the control.

5. Sterile fresh egg yolks
6. Streptomycin (or dihydrostreptomycin)

The sterilized agar is cooled to 55°C. Twenty-five ml. of egg yolk is added to each 75 ml. of medium. The flask is stoppered and shaken. Then the proper concentration of streptomycin is added to each of three flasks. The concentrations employed are, respectively, 1.0, 10, and 100 γ per ml. of medium. A stock solution, from which the necessary dilutions can be made, may be prepared by adding 10 ml. of distilled water to 1.0 Gm. of streptomycin.

After thorough mixing, the medium is dispensed aseptically into tubes or bottles and allowed to harden in the desired position. It has been found that the small flat-sided bottles with bakelite screw caps (in routine use in this institution for many years) are more practical for this type of work than tubes. The bottles have a capacity of approximately 30 ml.; about 8 ml. of medium is placed in each. Since this medium is not autoclaved after the addition of eggs and streptomycin, the greatest caution must be observed in order to prevent contamination. The bottles of medium are incubated for forty-eight hours, examined for contaminants, then stored in the icebox until used; if not used within thirty days, they should be discarded.

B. Modified Lowenstein Medium: This is employed as an additional medium for purposes of isolation in the event that growth does not occur on the nutrient agar egg yolk medium.

Ingredients:

1. Mineral salt solution
   - Mono-potassium phosphate ..... 2.4 Gm.
   - Magnesium sulfate ..... 0.24 Gm.
   - Magnesium citrate ..... 0.6 Gm.
   - Asparagin ..... 3.6 Gm.
   - Glycerol ..... 12.0 ml.
2. Distilled water, added to make 600 ml. The crystals are dissolved by heating and the mixture is placed in an Arnold sterilizer for fifteen minutes. It is then cooled thoroughly.
3. Starch solution. Thirty grams of potato flour are placed in a sterile 1,500-ml. Erlenmeyer flask (Pyrex) containing 100 Gm. of sterile glass balls; this is mixed in the dry state. Then the mineral salt solution is added slowly and the whole is mixed thoroughly. The flask is placed in a boiling water bath and heated with constant agitation until a satis-

[2] The 2 per cent Malachite green solution should be made up at least 24 hours before using and incubated at 37.5° C. for at least 24 hours to facilitate dissolving.

factory paste is produced (usually in about 20 to 25 minutes). Finally it is heated in a 56° C. water bath for one hour.

4. Egg fluid. Enough cleaned sterile hens' eggs to make 1,000 ml. are broken into a sterile graduated cylinder, poured directly into the potato-flour-salt solution and mixed thoroughly by shaking.

5. Malachite green. Twenty milliliters of 2 per cent aqueous Malachite green is added. The whole is mixed thoroughly and filtered through sterile gauze directly into a sterile separatory funnel. The medium is then bottled and inspissated immediately at 85° C. for half an hour. It is allowed to remain in the inspissator overnight at room temperature, then inspissated the following day at 75° C. for half an hour.

Dispensing Apparatus

Although a separatory funnel may be used to dispense the nutrient agar egg yolk medium, it has been found that in the case of noninspissated media the additional exposure to air is likely to result in a higher number of contaminations. Consequently, a simple apparatus has been devised which can be fitted to the mouth of the medium flasks and through which the medium can be introduced directly into bottles. This consists of a rubber stopper in which two or more holes are bored; one for the air inlet, and one or more for the dispensing taps. The air inlet, a glass tube reaching nearly to the bottom of the flask when the stopper is inserted, is bent at a sharp angle about two inches above the stopper so that when the flask is inverted the air inlet tube will curve upward. The dispensing taps extend into the flask less than 0.5 of an inch, and outside the flask are slightly curved away from each other and from the air inlet tube. On the projecting ends of all glass tubes are fitted short lengths of rubber tubing, attached at the other end to short pieces of glass. These latter are plugged with cotton before the apparatus is wrapped in paper and sterilized. After the medium has been prepared and when it is at the required temperature, this prepared stopper is fitted to the medium flask.[4] Pinch clamps are attached to the rubber tubes, and the flask is inverted and placed in a ring stand. The pinch clamp is removed from the air inlet, the cotton plugs are removed from the dispensing taps, and the taps are flamed. After the first few milliliters of media have been discarded, the bottles are filled, being flamed at the mouth before and after filling. Each dispenser may be used only once before being recleaned and sterilized, hence it is necessary to prepare as many dispensers as there are flasks of media in use.

Procedure for Streptomycin Sensitivity Tests

Preparation of sputum concentrates: The streptomycin sensitivity tests are made directly from 24-hour sputum specimens. The sputum is poured into sterile regulation sputum bottles of about 15 ml. capacity, and an equal amount of 3.0 per cent sodium hydroxide is added. If the sputum is unusually heavy and mucopurulent it may be necessary to use more sodium hydroxide. After cork stoppers are inserted, the bottles are shaken and then placed in the incubator (37.5°C.) for twenty-five minutes. About two minutes of shaking is sufficient for most specimens but watery ones will require less time; and extremely heavy mucopurulent ones, more time. Timing of treatment is an exceedingly important point, since overtreatment may render tubercle bacilli incapable of growth and undertreatment may cause an increase in contaminations.

[4] It is fastened in place by means of a heavy wire or metal "collar" which will encircle the neck of the flask and a metal loop which will be attached to the collar and hold the stopper firmly in position.

The bottles are removed from the incubator promptly. After one drop of 0.1 per cent phenolphthalein has been added, 2N HCl is added dropwise until the treated sputum is colorless. It goes without saying that the greatest caution must be observed in handling and shaking these specimens. The use of a hood is advised.

Each specimen is then poured into a sterile round-bottomed test tube, preferably Pyrex, which is plugged with sterile cotton held in place by a rubber band. The test tubes are centrifuged at 2,400 rpm for twenty minutes.

The supernatant fluid is decanted, leaving a few drops to facilitate mixing the sediment. Then the sediment is cultured on the media bottles, either using a standard wire loop and flaming before and after each inoculation, or else using individual sterile glass rods. The sediment should be cultured as soon as possible after centrifugation.

After the sputum has been cultured, remaining sediment is smeared thickly on a glass slide; the smear is dried, heat-fixed, and stained according to the Ziehl-Neelsen method. Results of the microscopic examination are incorporated in the records.

Culturing apparatus and method of inoculation: Because of the great number of specimens which must be cultured at this laboratory, the routine method of using a wire loop and a flame has proved too time consuming. Culture work is carried out in a glass-windowed chamber, and individual sterile glass rods or "spoons" are used to smear the sediment on the media.

The chamber is constructed of wood and metal, having a frame about 15 inches in depth and height, and 20 inches in length. As illustrated in figure 1, the top front is glass and the lower part of the front contains two armholes fitted with short sleeves. These are openings in the sides (A, B) through which the bottles may be pulled in and ejected from the chamber. Within the chamber are placed a rack for holding the test tubes, a holder for the test tube from which smears are being made, and a rack for holding sterile glass rods.

For each sputum specimen five bottles are labeled: the Löwenstein medium, and the four bottles of nutrient agar egg yolk medium consisting of a control and the bottles containing respectively 1.0, 10, and 100 γ of streptomycin per cc. of media.

The chamber is prepared about twenty minutes in advance by spraying with propylene glycol[5]; the cloth-covered openings at (A) and (B) are thoroughly moistened with 5 per cent phenol. Just before the culture work is begun, the test tubes and sterile glass rods are placed in appropriate racks. The labeled media bottles are placed in order in the "chimney" and each is, in its turn, pulled into the chamber by means of a lever through a slot. The first test tube is then placed in the holder, a glass rod is inserted and twirled rapidly about to mix the sediment thoroughly. Then each of the five bottles is inoculated with the sediment, using the same rod, and each is recapped and ejected through the opening at (B). Care must be taken to smear the same amount of sediment on each bottle of medium. After inoculation of one specimen, the test tube is replaced in the rack and the next placed in the holder and treated in the same manner, using a new glass rod. If the work is carried out properly, no flaming is required.

The glass rods used in culturing are about 15 cm. in length. The shallow spoon at the tip is made by heating one end of a rod in a bunsen burner flame until a knob has formed, then indenting this with a heavy wire about 0.5 cm. in diameter. With a little practice as many as 100 of these rods can be made in an hour, more than enough to cover the number of specimens cultured at any one time thereafter.

The rods are cleaned, wrapped in paper or placed in a glass container, and sterilized. When the rods are used for culture work, round-bottomed test tubes must be used to hold the sputum since the rods would not reach into the tapering end of a centrifuge tube.

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[5] Or the chamber may be fitted with an ultraviolet lamp.

The recording and reporting of streptomycin sensitivity tests: The tests are read at two-, three-, and four-week intervals and are reported as soon as conclusive results are obtained. Doubtful cultures are left for further growth until the fifth week. The cultures may be held longer than this, but only for diagnostic purposes.

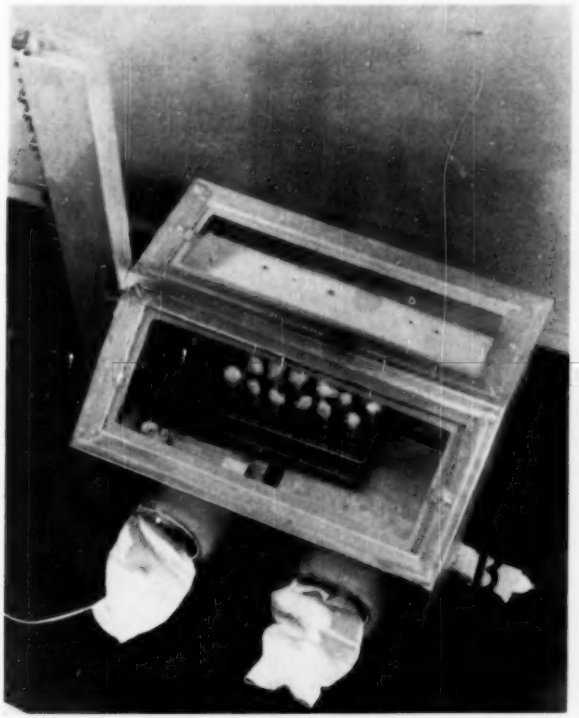

FIG. 1. Culturing apparatus

The amount of growth is recorded in the following manner:

1. 4+ Profuse confluent growth
2. 2+ Slight to moderate confluent growth
3. 1+ Partially confluent growth and isolated colonies, or isolated colonies too numerous to count (over 100)
4. Less than 100 colonies (actual number of colonies recorded)

In order to determine roughly the relative numbers of resistant and sensitive tubercle bacilli, the amount of growth present in medium containing streptomycin is compared on the above basis with the amount of growth on the con-

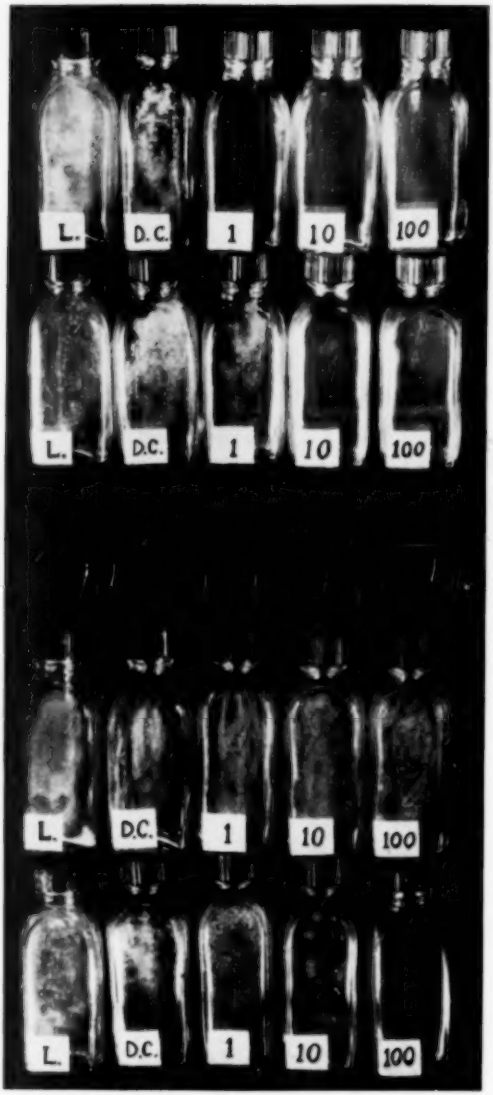

FIG. 2. Results of streptomycin sensitivity tests on four sputum samples.
Top row: Sensitive to (less than) 1.0 γ per ml.
Second row: Partially resistant to 1.0 γ per ml.
Third row: Completely resistant to 100 γ per ml.
Bottom row: Slightly resistant to 10 γ per ml.
L = Löwenstein's medium control
DC = Nutrient agar egg yolk medium control

trol medium. Readings are made in terms of the highest concentration of streptomycin to which the tubercle bacilli are resistant (figure 2), and the cultures are reported as completely resistant (CR), partially resistant (PR), slightly resistant (SR), very slightly resistant (VSR), or sensitive.

Results of the direct streptomycin sensitivity tests are not considered valid when: (*1*) contaminations interfere with the readings; (*2*) the growth is irregular (for example, growth only on the ten γ bottle or only on the Löwenstein medium); (*3*) the growth is sparse with so few colonies as to make the readings unreliable. In such cases sensitivity tests are repeated, using a suspension prepared from all colonies on the control bottles. Final readings may be made in three weeks. It has been found that streptomycin sensitivity of any sputum culture showing very sparse growth is likely to be unreliable even if the original test is repeated.

TABLE 1

Analysis of Data of Direct Streptomycin Sensitivity Tests

	SPUTUM SPECIMENS TESTED	
	Number	Per cent
Number of specimens cultured	2,009	100.0
Number of specimens from which tubercle bacilli were cultured	1,328	66.1
Number of positive cultures on which streptomycin sensitivity was determinable in two to four weeks	914	68.8*
Number of positive cultures showing five or fewer colonies per bottle of medium	190	14.3*
Number of direct sensitivity tests showing irregular growth	100	7.5*
Number of cultures contaminated	124	9.3*

* Per cent of sputum specimens positive on culture.

One colony, for example, is hardly representative of the total population of tubercle bacilli in the body, and the sensitivity or resistance of that one strain is not necessarily indicative of the total sensitivity or resistance. For this reason the significance of the results of all repeated sensitivity tests made from ten colonies or less should be interpreted with caution. In tests made directly from sputum, all tests in which there are only five colonies or less per tube of medium are arbitrarily repeated.

RESULTS

In table 1 are shown the results obtained with the direct sensitivity test method on 2,009 specimens of sputum. This number of specimens represents samples obtained from approximately 800 patients.

It was possible to determine directly in two to four weeks the sensitivity to streptomycin of 68.8 per cent of the specimens from which tubercle bacilli were isolated. The remaining 31.2 per cent were accounted for by the cultures of fewer than five colonies (14.5 per cent); those showing irregular growth, including those in which the Löwenstein's showed growth and the nutrient agar egg yolk

did not (7.5 per cent); and cultures which were contaminated. The apparently large number of the contaminated cultures (9.3 per cent) does not represent the actual per cent of contaminations of all bottles of media, for a single contaminated bottle out of the five may invalidate the entire test.

Streptomycin sensitivity tests had to be repeated on approximately 31 per cent of the sputum specimens. On first glance this number seems large. When it is considered, however, that sensitivity to streptomycin was determined on more than two-thirds of the cultures at the time of primary isolation and that, had this not been done, all of these would have been recultured to determine the sensitivity, the saving in time and labor may be better appreciated. Actually the performance of streptomycin sensitivity tests on tubercle bacilli at the time of isolation has resulted in a tremendous saving of time and labor in this laboratory. Most important, the method permits an evaluation of bacillary sensitivity or resistance *while the drug is being given.*

COMMENT

The efficiency of this method depends upon the fact that the majority of patients in this series were excreting relatively large numbers of tubercle bacilli. Should the relative number of such strongly "positive" patients be reduced, it is possible that the direct sensitivity testing method would entail more labor and loss of time than repeating the tests from diagnostic cultures.

The possibility is recognized that the medium employed for direct sensitivity tests may not be as suitable for isolation of tubercle bacilli from sputum, because of the presence of agar, as some of the inspissated egg yolk media. Nevertheless, the nutrient agar egg yolk medium in this laboratory has actually compared quite favorably with the modified Löwenstein medium. The inclusion of streptomycin in any inspissated medium, on the other hand, necessitates taking into account the destruction of streptomycin by heat. The amount destroyed may be as much as 55 per cent (3). This means that an excess amount of streptomycin must be added to the medium before inspissation so that the final concentration will approximate that desired. Since the amount of streptomycin destroyed will depend upon the time and temperature of inspissation, the concentration of streptomycin and the composition of the medium, it is difficult to see how, except under rigidly controlled conditions, the loss could be uniform even in different batches of medium in the same laboratory, much less between different laboratories. Therefore, accurate determination of the concentration of streptomycin in each batch of medium following inspissation would be necessary. This would pose a formidable, if not impossible, problem for many laboratories.

Apart from the above-mentioned considerations, no advantage would be gained by using an inspissated egg yolk medium. Such a medium might detect tubercle bacilli in a slightly higher percentage of specimens, but these would be those sputum specimens containing very small numbers of organisms. It was pointed out previously that the results of streptomycin sensitivity tests of such cultures are unreliable.

Inoculation of each sputum concentrate on to a suitable inspissated egg yolk medium at the same time that the streptomycin egg yolk agar is inoculated serves to reduce to a minimum the possibility that "positive" sputum will be overlooked.

SUMMARY

A method is described for performing streptomycin sensitivity tests on tubercle bacilli by culturing sputum concentrates directly on solid medium containing streptomycin.

Using a nutrient agar egg yolk medium, 2,009 specimens of sputum were cultured. Growth of tubercle bacilli was obtained from 1,328 and of these the sensitivity to streptomycin was determined on 68.8 per cent in two to four weeks. The results of the tests on the remaining 31.2 per cent were invalidated because of scanty growth, irregular growth, or contamination.

Use of the method has resulted in a marked reduction of the time usually required for completion of the tests; it has been more economical of labor and materials, and has been of great value to clinicians in controlling treatment.

SUMARIO

Método Directo para la Determinación de la Sensibilidad de los Bacilos Tuberculosos a la Estreptomicina

El método descrito permite ejecutar pruebas de sensibilidad a la estreptomicina en bacilos tuberculosos cultivando concentrados del esputo directamente en medio sólido que contenga dicha droga.

Usando un medio Difco dehidratado de yema de huevo y agar nutriente, cultiváronse 2,009 ejemplares de esputo. Obtuviéronse colonias de bacilos tuberculosos de 1,328, y de éstas se determinó la sensibilidad a la estreptomicina en 68.8 por ciento en término de dos a cuatro semanas. El resultado de los ensayos en el 31.2 por ciento restante fué inutilizado por virtud de insuficientes colonias, proliferación irregular o contaminación.

El empleo de esta técnica ha logrado decidida disminución del tiempo requerido habitualmente para terminar las pruebas; también ha economizado trabajo y materiales y resultado de gran valor para clínicos en la dirección del tratamiento.

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PLATE METHOD FOR THE DETERMINATION OF STREPTOMYCIN SENSITIVITIES OF MYCOBACTERIUM TUBERCULOSIS[1]

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INTRODUCTION

A simple method to test the drug sensitivity of *M. tuberculosis* is to streak the organisms on a solid egg agar plate containing various concentrations of streptomycin or *p*-aminosalicylic acid. The inhibition of the growth of organisms can then be measured by counting the number of colonies present at a particular concentration of the drug.

Many proposed methods for determining sensitivities have been tried in this laboratory and the surface growth on solid media is regarded as the most satisfactory measurement of the number of resistant organisms present in an inoculum (8). Liquid media such as Dubos-Davis Tween albumin and Youman's have given comparable results to the solid media in this laboratory and others (table 1) (7). It is believed that by using liquid media, when few resistant organisms are present, the results may be misleading, especially if the inoculum contains a majority of sensitive organisms. Liquid media methods are also more liable to contamination; and use of penicillin as an inhibitory agent to other organisms in liquid media has not proved satisfactory. Accordingly, the purpose of this paper is to describe a method for the determination of the streptomycin sensitivity of tubercle bacilli by the use of solid media.

OBSERVATIONS

Methods: The medium (Löwenstein-Jensen) is poured into a Petri dish, in 12 to 15 cc. amounts. Our laboratory is using the screw-top test tube, 15 mm. by 150 mm., for these determinations as well, but the tubes are difficult to clean upon completing the tests. By using the Petri dish, standard size, the solid egg media can be easily removed with a spatula after autoclaving.

The lids of the Petri dishes can be removed, and studies in colony morphology, bacterial dissociation, or animal inoculation can be made, if desired, thus giving a greater access to any particular colony.

This method also allows more surface area for inoculation and growth. The tubercle bacillus, being an obligate aerobe, will give heavier growth under these conditions than on an egg yolk agar slant (4). The lens of a Quebec colony-counter is used to count the number of colonies present at any given concentration of streptomycin or *p*-aminosalicylic acid incorporated in the media.

Specimens of sputum and gastric washings are concentrated by the trisodium phosphate method (1). Primary isolation of *M. tuberculosis* from sputum, gastric lavage, or urine is made on Löwenstein-Jensen medium (5, 6). Approximately 0.1 ml. of the concentrate

[1] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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TABLE 1

*Comparison of Results of Streptomycin Sensitivity Studies in Tween Albumin Medium and Löwenstein-Jensen Solid Egg Medium**

Sensitivity to streptomycin after 14 days' incubation (in units per cc.).

CULTURE NUMBER	TWEEN ALBUMEN MEDIUM	LÖWENSTEIN-JENSEN MEDIUM
903	>(100)	>(50) 10% growth at 50 5% growth at 100
992	>(10)	>(10) 1% growth at 50
906	<(2)	<(2) No growth at 2
848	<(2)	<(2) No growth at 2
195	>(100)	>(100) 25% growth at 50 10% growth at 100
743	>(2)	<(2) No growth at 2
601	>(10)	>(10) 50% growth at 2 12% growth at 10
29	<(2)	>(2) 10% growth at 2
56	<(2)	<(2) No growth at 2
69	>(100)	>(50) 15% growth at 50 5% growth at 100
35	<(2)	>(2) 1% growth at 10
36	>(2)	>(2) 10% growth at 2
908	<(2)	<(2) No growth at 2
903	>(100)	>(50) 10% growth at 50 5% growth at 100
844	>(20)	>(20) 15% growth at 20 3.0% growth at 50
795	>(2)	<(2) No growth at 2
907	>(10)	>(10) 50% growth at 10
20	<(2)	<(2) No growth at 2
120	>(2)	>(2) 10% growth at 2
68	>(2)	>(2) 1% growth at 2
75	>(10)	>(10) 1% growth at 50
25	>(100)	>(100) 25% growth at 100
986	<(2)	>(2) 15% growth at 2
695	>(2)	<(2) No growth at 2
579	>(2)	>(2) 1% growth at 2
57	>(2)	>(10) 10% growth at 10
60	>(2)	>(2) 10% growth at 2
173	>(100)	>(100) 75% growth at 100
119	>(10)	>(10) 100% growth at 10
108	>(100)	>(100) 100% growth at 100
88	>(2)	>(10) 100% growth at 10
77	>(100)	>(100) 25% growth at 100
76	>(2)	<(2) No growth at 2
61	>(2)	>(2) 5% growth at 2
996	>(2)	>(2) 10% growth at 2
45	>(20)	<(100) 100% growth at 50
52	>(100)	>(50) 5% growth at 100
37	<(2)	<(2) No growth at 2
39	>(2)	<(2) No growth at 2
45	>(20)	<(100) 100% growth at 50

* Data incorporating *p*-aminosalicylic acid sensitivity tests are not included as they are not complete at the present time.

is inoculated on the solid egg yolk media. Cultures are incubated at 37°C. and examined weekly; an average of four weeks being required to obtain sufficient growth. Positive cultures are removed and are subcultured with a stiff wire loop into Dubos-Davis Tween albumin medium in order to obtain a diffuse growth (3). When inoculating the liquid medium, as many colonies from the Löwenstein-Jensen medium as possible are used in order to obtain a representative sample for resistance studies. The subcultures on Dubos-Davis medium usually grow well in several days and some even within 72 hours, depending on the size of the inoculum and the "eugonic" nature of the organisms inoculated.

The Dubos culture is then centrifuged and the supernatant fluid poured off aseptically. The sediment is then used as the inoculum. This aids in obtaining the heavy inoculum which is necessary for sensitivity studies. One large loopful of this sediment is inoculated by the streak method on Löwenstein-Jensen media in a Petri dish containing 0, 2, 10, 50, 100 γ of streptomycin per ml.; in the case of *p*-aminosalicylic acid 0.1, 1.0, 10, and 25 γ per 100 cc. of the media are used (6). One plate is used for each concentration and culture studied.

The plates are then incubated for 14 days at 37°C. and the reading recorded. Sufficient growth may occur in several days, or in some instances the plates are held 21 days for final reading. A longer period of time is not considered necessary by our methods as no further change in sensitivity readings has been noted after 21 days other than more growth, equally at each concentration, as previously recorded at 21 days.

A comparison of the results of streptomycin sensitivity studies in Tween albumin and Löwenstein-Jensen solid (egg) medium may be seen in table 1.

Cultures of tubercle bacilli isolated from treated cases were found to be uniformly sensitive or resistant in Dubos-Davis Tween albumin and Youman's liquid medium as compared with Löwenstein-Jensen solid medium containing varying concentrations of either streptomycin or *p*-aminosalicylic acid. The solid medium technique provides a more quantitative reading as shown in our method of reporting (2). With solid egg medium, inhibition of contaminating bacteria with Malachite green is more satisfactory than the use of penicillin on contaminants in liquid media.

Direct sensitivity determinations without primary isolation have not proved satisfactory in the treated cases at this hospital and primary isolation of *M. tuberculosis* is considered the best procedure to follow prior to actual sensitivity testing.

The Petri plates containing Löwenstein-Jensen egg yolk agar allow more surface area for growth by satisfying the obligate aerobic requirements of *M. tuberculosis* which allows sufficient growth in 14 or 21 days. The lids of the Petri plates can be removed, thereby giving greater access to growth from which further studies can be performed on any particular colony with respect to morphology, bacterial dissociation, or animal inoculation. Upon completion of the readings, the Petri plates are easily cleaned.

SUMMARY

A technique has been developed in which a Petri dish was used for the determination of streptomycin sensitivities of *Mycobacterium tuberculosis*.

An estimate of the number of resistant organisms in a particular culture may be noted better by the use of the Petri plate than with the test tube growth using solid egg media. The Quebec colony-counter is a helpful aid in giving a

more accurate count when using the plate method. Thus, the per cent of growth at a given concentration of a drug can be compared with the growth on a control plate.

SUMARIO

Técnica de la Placa para la Determinación de la Sensibilidad a la Estreptomicina de los Bacilos Tuberculosos

La técnica elaborada utiliza una placa de Petri para determinar la sensibilidad del *Mycobacterium tuberculosis* a la estreptomicina.

Puede computarse mejor el número de gérmenes resistentes en un cultivo dado utilizando la placa de Petri que con las colonias del tubo de ensayo usando medios sólidos de huevo. El cuentacolonias Quebec constituye un auxiliar útil por facilitar cuentas más exactas, cuando se emplea la técnica de la placa. Por ejemplo, puede compararse así el porcentaje de colonias a una concentración dada de una droga antibiótica o bacteriostática con las presentes en una placa de comprobación.

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A COMPARISON BETWEEN AN ANEROID SPIROMETER AND A WATER SPIROMETER[1]

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Measurements of the amounts of respired air are of interest in clinical medicine because of their application in the study of cardiopulmonary function in health and disease. One of the simplest of these measurements is the vital capacity. A close relationship between the dyspnea of patients with either cardiac or pulmonary abnormalities and the vital capacity has long been established (1). In addition, numerous workers have shown how the effects of therapy can be followed by this method. For many years a somewhat cumbersome water-displacement air cylinder about eight liters in volume has been employed for the determination of the vital capacity. Because of the difficulty in transporting this apparatus, efforts have been made to develop a small aneroid type of spirometer. The purpose of this report is to show the results obtained with an experimental aneroid spirometer as compared with those noted on a standard water-displacement model in a large series of normal subjects and patients with pulmonary disease.

MATERIAL AND METHODS

The series included adult male and female patients, and normal subjects between the ages of 23 to 55 years, selected at random from the wards, outpatient department, and hospital personnel of the Division of Pulmonary Diseases of Montefiore Hospital. The patients all suffered from pulmonary tuberculosis and had undergone various forms of surgery such as pneumonectomy, thoracoplasty, or extrapleural plombage; other patients were being treated with either unilateral or bilateral pneumothorax. Some of the patients were on strict bed rest, others were ambulatory or semi-ambulatory. No effort in this study was made to compare the vital capacity of the different groups of patients or of the normal subjects.

The water-displacement type of spirometer employed was a standard-make unit, recently calibrated. The aneroid unit[2] used in this study is illustrated in figure 1. The component parts and the simple, sterile, cardboard mouthpiece which is discarded after use are shown in figure 2.

The experimental model is easily disconnected, and can be cleaned and made sterile with a detergent antiseptic, Raccol[3].

Both patients and normal individuals were instructed to take as deep an inspiration as possible and to expel all the air possible into the mouth pieces of the test units. To keep error at a minimum, the order of testing the two units was alternated.

[1] From the Division of Pulmonary Diseases, Montefiore Hospital, New York, N. Y.

[2] Imported Specialties Co., New York, are distributors of this unit.

[3] Raccol may be obtained from Winthrop Stearns Co., Inc., New York.

Two determinations with each instrument were made on all individuals. The dials in each instrument are calibrated in liters with 100 cc. divisions.

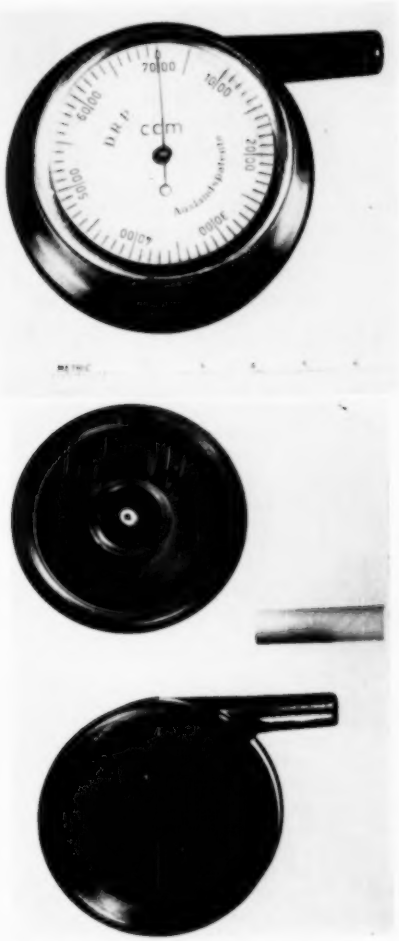

FIG. 1. (Top) Anterior view of the aneroid spirometer to illustrate appearance and size.
FIG. 2 (Bottom) This figure illustrates component parts of the spirometer with the detachable mouthpiece.

OBSERVATIONS

The results of duplicate determinations of the vital capacity in a series of 100 normal subjects and patients by means of the water and aneroid spirometers are shown in table 1. The data reveal a close correlation between the two methods.

TABLE 1

*Comparison of Water-displacement and Aneroid Methods for Determination of Vital Capacity**

NUMBER OF DETERMINATIONS	METHOD		NUMBER OF DETERMINATIONS	METHOD	
	Water	Aneroid		Water	Aneroid
	cc.	cc.		cc.	cc.
1	2,200, 2,100	2,000, 2,200	51	3,000, 3,050	3,000, 3,100
2	2,300, 2,400	2,700, 2,650	52	2,900, 3,000	2,800, 2,900
3	2,200, 2,100	2,000, 2,200	53	4,200, 4,000	4,000, 4,000
4	2,100, 2,200	2,300, 2,300	54	4,000, 3,900	3,900, 4,000
5	2,100, 2,200	2,250, 2,200	55	4,000, 4,000	3,800, 3,900
6	2,500, 2,750	2,500, 2,550	56	2,200, 2,200	2,250, 2,200
7	2,350, 2,250	2,250, 2,400	57	2,950, 3,000	3,000, 3,000
8	1,400, 1,400	1,350, 1,400	58	2,500, 2,500	2,500, 2,500
9	1,900, 2,100	2,300, 2,400	59	1,850, 1,800	1,800, 1,850
10	2,200, 2,300	2,400, 2,400	60	2,400, 2,400	2,350, 2,400
11	3,250, 3,200	3,100, 3,200	61	2,700, 2,600	2,700, 2,700
12	3,700, 3,850	3,700, 3,800	62	2,000, 2,050	2,100, 2,200
13	2,900, 2,900	2,900, 2,900	63	4,800, 4,800	4,900, 4,800
14	3,500, 3,550	3,500, 3,450	64	1,900, 1,950	2,000, 1,900
15	2,750, 2,800	2,700, 2,750	65	2,450, 2,400	2,400, 2,500
16	2,100, 2,100	2,050, 2,100	66	3,100, 3,100	3,100, 3,050
17	2,000, 2,100	1,900, 1,900	67	2,800, 2,900	2,900, 2,900
18	2,400, 2,350	2,450, 2,500	68	4,000, 4,050	4,000, 4,000
19	2,700, 2,700	2,600, 2,700	69	4,700, 4,700	4,650, 4,700
20	2,700, 2,800	2,750, 2,800	70	3,500, 3,600	3,500, 3,500
21	2,900, 3,000	3,150, 3,200	71	4,200, 4,150	4,100, 4,150
22	4,000, 4,050	4,100, 4,100	72	3,800, 3,800	3,700, 3,700
23	2,250, 2,350	2,400, 2,450	73	4,000, 4,050	4,100, 4,150
24	1,500, 1,600	1,650, 1,700	74	1,900, 1,850	1,900, 1,900
25	3,800, 3,800	3,800, 3,800	75	1,500, 1,500	1,550, 1,500
26	2,700, 2,700	2,600, 2,700	76	1,450, 1,400	1,400, 1,400
27	2,000, 2,100	2,050, 2,150	77	2,500, 2,500	2,450, 2,500
28	1,500, 1,600	1,400, 1,500	78	1,400, 1,400	1,350, 1,400
29	1,500, 1,500	1,600, 1,550	79	2,500, 2,450	2,450, 2,500
30	900, 900	850, 850	80	1,950, 2,000	2,000, 2,000
31	1,100, 1,050	1,100, 1,150	81	4,000, 4,000	4,100, 4,000
32	2,200, 2,100	2,000, 2,100	82	1,800, 1,850	1,900, 1,950
33	1,800, 1,750	1,800, 1,850	83	2,700, 2,600	2,600, 2,600
34	1,900, 1,850	1,800, 1,850	84	2,250, 2,300	2,300, 2,300
35	1,500, 1,550	1,600, 1,600	85	4,000, 3,900	4,000, 4,000
36	2,500, 2,450	2,450, 2,400	86	2,300, 2,350	2,250, 2,300
37	3,000, 3,100	2,900, 3,000	87	1,800, 1,850	1,800, 1,800
38	3,500, 3,500	3,400, 3,500	88	3,300, 3,200	3,200, 3,250
39	2,800, 2,900	2,800, 2,900	89	3,150, 3,200	3,100, 3,100
40	2,100, 2,100	2,200, 2,200	90	1,850, 1,900	1,900, 1,900
41	2,800, 3,000	2,900, 3,000	91	1,900, 1,950	2,000, 2,000
42	2,700, 2,650	2,700, 2,700	92	3,100, 3,100	3,000, 3,100
43	2,600, 2,700	2,600, 2,600	93	2,800, 2,800	2,800, 2,800
44	2,200, 2,150	2,300, 2,200	94	1,750, 1,800	1,800, 1,800
45	900, 1,000	1,000, 900	95	1,910, 1,900	1,850, 1,900
46	1,800, 1,900	1,800, 1,700	96	2,300, 2,400	2,500, 2,400
47	700, 700	800, 700	97	3,900, 3,950	3,900, 4,000
48	3,700, 3,750	3,700, 3,800	98	1,350, 1,400	1,400, 1,400
49	3,300, 3,300	3,250, 3,200	99	4,600, 4,700	4,700, 4,700
50	2,000, 2,100	2,000, 2,050	100	3,750, 3,700	3,700, 3,700

* Readings done in duplicate.

The average figure for the water displacement instrument of the entire series was 2,598 cc., whereas the average figure obtained for the aneroid type of unit was 2,609 cc. The individual comparative readings are more significant because little variation was noted in any single instance. It is of interest to note that the readings of one unit are not consistently higher or lower than those of the other.

SUMMARY

The relatively comparable and reproducible readings of the aneroid model and the standard water-type of spirometer suggest that the new aneroid unit is a reliable instrument. The small size, ease of use, and portability are additional features of the aneroid spirometer which are of value to the physician, in his office or at the bedside, in determining the vital capacity in patients with cardiopulmonary dysfunction.

SUMARIO

Comparación entre el Espirómetro Aneroide y el Hidro-Espirómetro

Las lecturas relativamente comparables y reproducibles del espirómetro aneroide y del corriente de tipo hidráulico indican que el nuevo aneroide es un instrumento digno de confianza. El pequeño tamaño, fácil empleo y portabilidad constituyen otras caracterestícas del espirómetro aneroide que resultan de valor para el médico, ya en su consultorio o a la cabecera del enfermo, al determinar la capacidad vital de los enfermos con disfunción cardiopulmonar.

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Editorial

Tuberculosis on the Navaho Reservation[1]

Though this discussion is based upon the findings of a Medical Team[2] investigating the health of the Navaho Indians for the American Medical Association, September 1948, a review of the recent literature[2-8] and conferences with physicians and nurses in the Indian Service indicate that the findings and recommendations of the Team with reference to tuberculosis are almost equally applicable to other North American Indians as they appear on their respective reservations. In spite of a gradually falling death rate from tuberculosis even among some of the Indian tribes, it may be said that relatively the Indian reservations stand as ominous reservoirs of infection.[5] Experience[2] indicates that this hazard to the general population, rendered more acute because of the increasing Indian population and the necessity of more contact with the outside world, is wholly unnecessary and could be eliminated by applying the advantages and controls under which the general population is achieving its manifest destiny. To a great extent this also applies to the Negro and Mexican contingents in the United States.

Tuberculosis on the Navaho Reservation is approximately ten times the average for the United States.[2] The death rate has been reported slightly above 300 per 100,000 compared to 30.0 in the general population. In recent years, with one or two exceptions, the turnover of medical personnel has been so rapid that physicians have not remained long enough to form a valid opinion of the situation. The records are so incomplete, so wanting in content and continuity that they supply no reliable consecutive statistical data on this question.[4] Nevertheless, tuberculin testing and mass roentgenographic surveys indicate widespread infection and a high incidence of manifest pulmonary tuberculosis.

Compared to the white population, the Indians studied apparently follow the accepted epidemiological pattern, and the disease conforms in character and behavior to that commonly found among the white population.[8] This applies not only to the pulmonary manifestations, but also to the lymphatic, bone, joint, intestinal, and other forms of the disease.

[1] A recent visit to the Navaho-Hopi Reservations indicates a gratifying improvement not only in the management of tuberculosis (fluoroscopic facilities having been supplied and collapse therapy resumed) but in the general medical and surgical services. The Chief Medical Officer and his staff deserve the highest praise for untiring efforts under great handicaps. This improvement is reflected in the recent report by Pijoon *et al.* (J.A.M.A., 1949, *140*, 1013).

[2] Health of the Navaho-Hopi Indians, General Report of the American Medical Association Team, J.A.M.A., 1949, *139*, 370.

[3] Braasch *et al.*, J.A.M.A., 1949, *139*, 220.

[4] Woods, O. T., J.A.M.A., 1947, *135*, 981.

[5] Myers *et al.*, Hygeia, 1947, *25*, 272.

[6] McGibony *et al.*, Am. Rev. Tuberc, 1945, *52*, 104.

[7] Winters, S. R., Hygeia, 1944, *22*, 680.

[8] Long *et al.*, Am. Rev. Tuberc., 1936, *33*, 407.

The over-all appraisal of the Navaho's seeming lack of resistance must take into account the fact that, according to accepted standards, the available food supply in the average hogan is seriously deficient in variety, quality, and quantity.[7] The daily dietary for the average Indian family would be considered decidedly inadequate for white people. It is difficult to think of a large group of American people virtually without milk and eggs, vegetables, fruits, and fruit juices; yet we have in the midst of plenty this Navaho island of sixty-four thousand people on a mere mess of pottage while boldly we feed the world. The average Navaho's chief source of subsistence is corn meal and mutton stew served the same way three times a day if he is fortunate enough to eat that often. He has squash, green corn, beans, and melons in season.

Regardless of its merits, the sheep- and goat-reduction program has materially cut the source of protein and fat in many of the Navaho families. It has been said that the average family formerly consumed one sheep a week. Now the margin is so narrow that often they conserve the flock and go hungry in order to have lambs and wool. In view of these facts and some of the newer concepts with reference to diet, may we not be justified in presuming a certain mutual adaptation between the Indian and his desert food which helps to balance and sustain the human economy? We are not fully informed as to the content of desert herbs, roots, seeds, and nuts consumed by the Indians. Perhaps devouring the whole sheep supplies certain elements otherwise absent and helps to stave off scurvy and beriberi, as on the Santa Fe Trail when the hunters, traders, trappers, and members of the caravan ate all of the buffalo sans only horns, hooves, hair, and hide. "On the prairie you will eat cow's insides for choice marrow, lights, heart and tongue, warm liver spiced with gall, and best of all, guts—plain guts—and raw at that!" The white man learned the value of this comprehensive consumption from the Indians. Who knows what vitamins and minerals these visceral organs may take from the desert herbage?

Considering all the environmental factors, it is surprising that the Navahos withstand the impact of this killing disease as well as they do. Since the presence of pre-Columbian tuberculosis on this continent has been established, perhaps the Indians have had more time for the development of racial resistance than is commonly realized. If, as believed by some, the Navaho's hybrid origin begets vigor and increased fecundity, may it not also supply a certain degree of resistance. Certainly there is reason to suspect that the Navaho's inability to conform to the average statistical levels for tuberculosis in the general population may be due to unfavorable environmental factors and inadequate medical service.[7] Following the flocks afoot with undiagnosed tuberculosis and coming home to the overcrowded hogan at night with only corn pone and mutton and a skin on the bare ground for a bed cannot be compared to resting in a sanatorium with the white man's full dietetic and hygienic management under expert medical direction.

After a general survey of the situation in the field, the writer visited the sanatorium located at Fort Defiance. Ninety-seven bed patients were seen and 8 or 10 were carefully examined. These examinations were for the purpose of

determining clinical manifestations and, as far as possible, the course of the disease in each case. Serial roentgenograms on approximately 50 cases, including those receiving a physical examination, were carefully studied. The accumulated evidence suggests that the relatively high incidence of tuberculosis and the high morbidity and mortality from this disease among the Navaho Indians are not a matter of racial susceptibility and innate lack of resistance, but are due to provocative environmental influences, including insufficient and improper food, inadequate health education, ineffective methods of case finding, insufficient beds for known cases, haphazard management, plus serious therapeutic deficiencies.

There are virtually no health, educational, or rehabilitation services at the sanatorium but the nursing, food, and housekeeping are apparently fairly adequate. The building, though not planned for tuberculous patients, is quite satisfactory, except for the fact that there are no facilities for the isolation of the desperately ill or troublesome cases. The patients are in wards of approximately twenty beds each. After allowing for the absence of fluoroscopic facilities, the equipment is sufficiently good to warrant adequate care for the average patient, presuming the availability of a conscientious, competent, professional staff. At the time of this inspection, collapse therapy was not being employed. A limited number of patients were receiving streptomycin.

In consideration of the above facts, it may be said that the patient has a good boarding house where he can come and go at will and where the cure and control program marks time while the Indians pay with their lives and the taxpayers with their hard-earned increment. The one medical sanatorium staff officer is not to blame for all the above therapeutic deficiencies. Though he were adequately qualified through training and experience, he would still need the benefit of consultants in various fields, including thoracic surgery and bronchoscopy, to meet the requirements of good routine management. Adequate care of the tuberculous can be achieved only through team work between physicians possessing all the diagnostic and therapeutic skills, technical knowledge, and experience necessary to encompass and negotiate the over-all task. Such a service on the Navaho Reservation is impossible under present conditions.

In the short time allotted and the absence of adequate clinic and hospital records,[4] which should exhibit informative diagnostic and therapeutic data, it is impossible to determine the relative incidence of other bronchopulmonary conditions. Reports from physicians and nurses indicate that the incidence of acute respiratory episodes, including the pneumonias, is at least as high as the average in the population outside the reservation. Apparently the nontuberculous conditions, acute or chronic, are not more common than might be expected among the whites under similar living conditions. Studies with reference to histoplasmosis and sarcoidosis are wanting. In the course of this investigation dealing specifically with the Navahos, it was deemed advisable to make the following recommendations. Because of the prevailing environmental conditions and the general high incidence of tuberculosis among the American Indians as

a whole, it is highly probable that similar measures would be of value in other reservations throughout the country. These recommendations take on added significance when we consider that the increasing Indian population, coincident with diminishing resources on the reservations, makes it necessary for an ever increasing number to seek employment off the reservations. As a consequence, the stage is set for a dangerous seeding of fertile soil with tubercle bacilli from these overfilled reservoirs.

The recommendations follow:

1. A comprehensive case-finding campaign in the hands of trained workers should be conducted.[5] This should include mass roentgenographic examination of every man, woman, and child, followed by a careful diagnostic study of all cases showing obvious roentgenographic evidence of disease in the lungs.

2. There should be adequate field coverage of the population,[3] including health centers, field nurses, social service and welfare workers, and a trained sanitarian. Nurses' aids and interpreters with special training should be drawn from the educated Navahos.

3. Sufficient sanatorium beds should be made available so that all active cases could be hospitalized as discovered. This would serve not only to provide proper management for the individual patient, but would also break his contact with others.

4. An intensive educational program should be organized in order to disseminate knowledge of prevention and cure and to check the present practice of leaving the sanatorium against medical advice. The records show that during the past twelve months 77 patients left the sanatorium against advice. Considering the average number in the Navaho family, this meant the renewal of contact with four or five hundred people; a heaving seeding in rich soil. It is impossible to estimate the ultimate cost of this lack of control in dollars and cents, suffering, and death.

5. A program of universal tuberculin testing and BCG vaccination of all who reveal no skin sensitivity to tuberculin should be set up. A less comprehensive BCG program among various tribes has been under way since 1935[9] and the results warrant an all-embracing plan. A large number of children in the Reservation schools were being tested and BCG-vaccinated under the direction of Dr. Joseph D. Aronson at the time of this survey. A preliminary statistical report from Doctor Aronson on tuberculin-positive cases is in keeping with the above estimates in regard to the prevalence and behavior of tuberculosis on the Reservation.

In the general report of the Team[2], it was recommended that the 150-bed hospital at the Fort Defiance Medical Center be converted for the care of tuberculous patients, thus making the present Medical Center a sanatorium center with provision for all types of collapse therapy. This would make available 250 beds. A recent mobile roentgen unit survey report indicates that out of 16,000 persons examined 1,859 exhibited evidence of presumably progressive pulmonary

[9] Aronson *et al.*, Extracts from Pub. Health Rep., 1947, *61*, 802.

tuberculosis. If these figures are accepted as representative of the whole Navaho population, they suggest the immediate need of approximately 4,000 beds.

In the light of the above recommendations, it may be said that the Navaho Indians are a placid, timid, trustful, intelligent, malleable race, awaiting only a frank and kindly manifestation of good faith on our part in return for relatively full cooperation on their part. It is doubtful that such understanding can ever be reached through physicians available under the present plan (Civil Service). Even though such physicians were better paid, with assurance of tenure, it is doubtful if they would aspire to such a career in human weal unless endowed with the missionary spirit so badly needed by all physicians and nurses in the Indian Service.

Paradoxical as it may seem, economy lies in immediate free spending. The demand is for the very best professional personnel and an expensive program; otherwise there can be no end to the present mounting costs in money, morbidity, and mortality.

The extensive program recommended implies a declining morbidity and mortality rate after the discovery and control peak is reached; therefore a diminishing demand for beds. The additional beds need not be of costly construction. A wise government will want to escape the criticism which might be aroused by the spectacle of a glittering empty therapeutic palace on the windswept desert. Another valid reason for inexpensive construction is the immediate need which otherwise cannot be met promptly.

Those who question the above implications should remember that the present opportunities for effective control are far superior to those presented to the National Tuberculosis Association, its allied organizations, and the Public Health Service soon after the turn of the last century. This is true of the Navaho Indians even after allowing for the linguistic, geographic, and residential difficulties on the Reservation. An intensive comprehensive attack will require money, adequate trained professional personnel, and a free hand on the part of the Chief Medical Officer, to accomplish the task; but effective control will pay dividents not only for the patient Indians who have waited so long but for the people of the nation who have a right to demand reasonable protection. Considering the acute forms of tuberculosis so often encountered among the Navaho Indians, may we not hope that ultimately chemotherapy will come to our rescue and help reduce the demand for long-continued confinement in sanatorium beds—what a boon to a primitive, undisciplined race accustomed to the chase but not the cure.

At present, the Navaho Reservation with this high tuberculosis mortality rate serves as a focus of infection for those who have spent a lifetime fighting to free their children and their children's children from the one-time "captain of the men of death." As previously indicated, this statement applies to other reservations.[5] After many years of haphazard medical care, the government should clean up these tuberculosis "hot spots."

It is estimated that with the most hopeful results of the stock reduction, irrigation, and soil conservation programs, the land cannot adequately support

more than 35,000 Navahos. This means that approximately 30,000 should immediately seek gainful occupations in the United States marts of commerce or in off-the-Reservation agricultural and other pursuits. Unfortunately, less than 15,000 Navahos speak English. In the last analysis, medical efficiency, Indian welfare, and the safety of the general public hinge upon the speeding up of general education and adequate tuberculosis control, not only among the Navahos, but throughout the North American Indian reservations.[3]

LEWIS J. MOORMAN

BOOKS

S. Lyle Cummins: *Tuberculosis in History. Pp. 205, The Williams & Wilkins Company, Baltimore, 1949, Price $4.50.*

Tuberculosis was in history long before Professor Cummins came upon the scene. But very properly he begins this interesting narrative early in the seventeenth century. The study is based upon biographical sketches of physicians whose critical curiosity regarding the disease drew them into the historical current and put the then undiscovered tubercle bacillus on the defensive. The treatise is divided into three parts. The first deals with British physicians who made valuable contributions; the second considers great advances made by the continental students of the disease; and the third presents interesting sketches of Edward Livingston Trudeau and Robert Koch, thus spearheading the modern era which in spite of all diagnostic and therapeutic progress finds tuberculosis still making history.

The author employs good historical sense when he declines to discuss the living workers and leaves them in the crucible until the fires burn low and time declares their ultimate worth. Through the interesting sketches of Trudeau and Koch the reader gets a touch of the modern without historical indiscretion since both achieved ineradicable rating in their respective spheres while still living.

Professor Cummins introduces Part I by discussing the life and works of the British physician Christopher Bennet (1617–1655) who died of the disease which engaged so much of his time and thought. His work on *The Nature and Cure of Consumption* is quoted in order to show his practical views on the disease in the midst of the general confusion. Cummins then turns to Thomas Willis (1621–1675). Though better known to students for having described the circle of Willis, he did important works on the anatomy and physiology of the lungs and, as the author points out, he wrote interestingly about "consumption arising through a fault of the lung" and, perhaps wisely, he said "in the lungs rather than in the heart or the brain the threads of life are spun and there they are oftenest broken." The story runs from Willis to Thomas Sydenham. The author fortifies his theme by calling to the attention of his readers Sydenham's famous statement, "Medicine depends on the observation of, not on hypotheses about, disease." Quoting from Sydenham's works, the author stresses his enthusiasm and recounts his belief in the efficacy of conservative horseback riding in the cure of consumption.

This ingenious method of revealing progress is followed throughout the book. It is not possible in a brief review to discuss in detail the life and work of each contributing individual. Suffice it to say that the first part not only deals with Bennet, Willis, and Sydenham but similarly with Benjamin Morten, William Stark, James Carson, Goerge Bodington, and William Budd. These names are familiar to those having even a meager knowledge of tuberculosis. Particularly is this true of Morten, Carson, and Bodington. The impact of this British group upon the disease, tuberculosis, brought to the world a better understanding of this deadly enemy of mankind and developed a more effective strategy against its methods of attack and its disastrous course. Its nature, its symptoms, its

pathology, its communicability, and its management were being conceived and recorded in preparation for further advances and ultimate confirmation of many of these dawning concepts.

In Part II the author deals with that well-known European group including Leopold Auenbrugger, Jean Nicholas Corvisart, Gaspard Laurent Bayle, René Théophile Laennec, Pierre Adolphe Piorry, and Jean-Antoine Villemin.

Beginning with Auenbrugger (1722–1809) who came tapping on the thorax, thus giving to the world his *inventum novum*, soon the reader's attention is called to the intriguing story of Corvisart's erudition and professional skill plus his reputation as Napoleon's physician, which enabled him to revive and popularize this important, though neglected, invention through a new French translation of Auenbrugger's work on percussion. This service, together with Bayle's publications stressing the importance of pathology in clinical pursuits, prepared the way for Laennec's invention of the stethoscope and his remarkable career and unprecedented influence upon medical progress, particularly in the field of tuberculosis.

Piorry and his pleximeter are presented and finally all advances and resulting concepts were to a great extent clarified by Villemin's proof of communicability by animal inoculation. The interesting and convincing account of his experiments was presented before the Academy of Medicine in 1867, only fifteen years before Robert Koch discovered the tubercle bacillus. The skillfully chosen quotations from the original writings of these indefatigable workers gives the story an unwonted ring of authenticity.

In Part II the interesting biographical treatment of Trudeau and Koch exhibits original charm and appeal. These stories are well known but re-reading is rewarding.

With Professor Cummins as coordinator and reporter, these makers of history in three eventful centuries are permitted to speak for themselves. Though this is not stressed by the author, the story is doubly interesting to the student of tuberculosis because so many of the workers were suffering from the disease while they were searching relentlessly for its secrets.

The book should be useful as collateral reading for students in history of medicine courses and every physician, regardless of his special interests, should be familiar with its contents.

LEWIS J. MOORMAN

RUTHERFORD T. JOHNSTONE: *Occupational Medicine and Industrial Hygiene. Pp. 604, The C. V. Mosby Company, St. Louis, 1948, Price $10.00.*

This is a very sound and comprehensive textbook and bears evidence of the author's wide knowledge of his subject and his many years of experience in the field.

In Part One, Dr. Johnstone discusses industrial medicine of the past and of the future. Workmen's compensation, the medico-legal aspects of industrial medicine, methods of diagnosis of occupational diseases, and the laboratory procedures incident thereto are all included.

Parts Two and Three consist of a thorough consideration of solvents and

metals. The signs and symptoms associated with exposure to these substances, as well as diagnoses and medico-legal aspects, are all featured.

Part Four consists of a detailed discussion of pulmonary dust diseases and the relationship of tuberculosis to occupation. Also included are chapters on the dermatoses, the synthetics, special industrial processes, and the control of environmental conditions, as well as individual protection against various hazards. Part Four concludes with an adequate discussion of pre-employment physical examinations.

In addition there is a valuable appendix listing the common trade names of products used in industry with their chemical composition.

This book is valuable both as a reference manual and as a textbook. It is attractively printed on good paper and the illustrations have been selected with care.

A. J. Lanza

David Zacks: *Photoradiography in Search of Tuberculosis. Pp. 295 with 250 illustrations, The Williams & Wilkins Company, Baltimore, 1949. Cloth, Price $5.00.*

The material covered in this volume has been greatly needed by physicians interested in tuberculosis and its control and also by the general practitioner. Over twelve million people are being X-rayed annually by photofluorographic units in our country and in every case the positive and suspicious findings are being referred to the family physician for follow-up studies and a thorough clinical examination to establish a diagnosis. Unfortunately, not all physicians see eye to eye on the purpose behind, or the value of, such methods of tuberculosis control. Some are even outspoken in their denunciation of mass X-rays, saying they are pure unadulterated socialized medicine; this, in spite of the fact that the sole object of the surveys is to place the suspected or known case under the care and supervision of his own physician as soon as possible.

Twenty years of careful service and observation went into the creation of this volume, which carries the reader through all phases of mass chest radiography. Since organization of the community is a *sine qua non* to a successful outcome, Zacks spends a considerable portion of the text on this phase of the problem. Procedures used in Massachusetts are outlined in detail and, while this reviewer believes that some of these details would be impractical in other areas such as a large rural state, they are nevertheless very informative and lend authority to the text. Choice of personnel, equipment, and problems of administration are presented in detail and with clarity, being aided by very fine illustrations. The pitfalls of reading small film, including over-reading and under-reading, are discussed.

Zacks states that the success of chest surveys will depend largely upon the good will and wholehearted cooperation of medical practitioners. This is a grave responsibility indeed and deserves serious consideration. Absence of symptoms does not exclude pulmonary tuberculosis. The limitations of the stethoscope should be clearly recognized. In the absence of symptoms and physical signs, a

subject referred by the small-film reader to his family physician as a result of suspicious small-film finding should not be dismissed as having a normal chest without having further X-ray study. Early pulmonary tuberculosis is usually clinically silent. Management of pulmonary tuberculosis according to the roentgenographic pattern is described. We regret that the tuberculin test is treated so sketchily; it is not even mentioned in the index.

The role of the diagnostic chest clinic, the duration of follow-up, and planning for such follow-up is handled adequately. A very well-executed portion of the text covers promotional and educational material to be used in conjunction with the surveys. It clearly defines the scope and work of the various community and voluntary agencies, school teachers, nurses, speakers, canvassers, and publicity personnel.

The highlight of the book, to this reviewer, is the excellent atlas of chest radiographs including serial film studies on minimal pulmonary tuberculosis. Other books on this subject have presented reproductions of 14 by 17 films. Where this volume excels is in the presentation of the actual small-film reproduction, which alone can reproduce the problems encountered in this work. Adequate descriptions accompany all radiographs. A very interesting feature is the reproduction of artefacts, clothing, buttons, contents of pockets, et cetera, which are easily confused with significant pulmonary shadows.

The volume is beautifully printed and published and is very reasonably priced. A fitting foreword has been written by Ezra Bridge. We are sure this book will never achieve the popularity it deserves. In our opinion, it should be read by every practicing physician.

F. Kenneth Albrecht

THE AMERICAN REVIEW OF TUBERCULOSIS ABSTRACTS

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CLINICAL STUDIES. TUBERCULOSIS

Intratracheal Rupture of Tuberculous Lymph Node.—A child aged six years who was suffering from tuberculosis suddenly developed fatal asphyxia in the presence of the authors. Necropsy showed perforation of a caseous lymph node into the trachea. This is a rare phenomenon.—*Fatal asphyxia from intratracheal rupture of a tuberculous mediastinal lymph gland, H. Angelman & W. M. L. Turner, Lancet, November 5, 1949, 2: 842.—(A. G. Cohen)*

Miliary Tuberculosis.—Text books rarely mention the time interval elapsing between a miliary spread and the earliest roentgenographic manifestation in the lungs. Observations made in 4 patients who clinically were suspected of developing a miliary spread indicate that at least fourteen days elapse before evidence of the spread can be ascertained roentgenographically.—*Zur Frage des frühesten Zeitpunktes der röentgenologischen Manifestation einer Miliar-Tuberkulose nach stattgehabter Aussaat, G. Sude, Tuberkulosearzt, 1949, No. 1: 35.—(E. Dunner)*

Tuberculin Sensitivity in Primary Tuberculosis.—In 200 cases of primary tuberculosis the threshold of tuberculin sensitivity was 1:1,000 in 4.5 per cent of the cases, 1:10,000 in 44 per cent, 1:100,000 in 38.5 per cent, and 1:1,000,000 in 13 per cent. Among 191 children reacting to 1:10,000 there was a weak reaction in 70.1 per cent, a moderate reaction in 26.8 per cent, and a highly positive reaction in 3.1 per cent. The tuberculin sensitivity in children with recent primary tuberculosis was similar to that of adults with tuberculous lesions of limited extent, which is considered the only comparable group. Tuberculin sensitivity was higher in the age group of 10 to 16 years than in younger children. No correlation was noted between sensitivity and the length of time elapsed since conversion. After tuberculous pleurisies, tuberculin sensitivity was found particularly low; it was rather high in tuberculous adenopathies of considerable extent. This study does not confirm the concept of a particularly high general allergy inherent in the phase of primary infection.—*Étude quantitative de la sensibilité cutanée à la tuberculine après la primo-infection de l'enfant, J. le Melletier & C. Le Prestre, Semaine d. hôp. Paris, June 22, 1949: 1972.—(V. Leites)*

Liver Biopsy.—Routine liver biopsy in patients with a roentgenogram suggestive of miliary tuberculosis may confirm the diagnosis of tuberculosis by demonstrating specific tuberculous lesions. The procedure facilitates the diagnosis of hematogenous dissemination since the liver was normal in bronchogenic dissemination of the miliary type. The method may be helpful in the differential diagnosis of miliary carcinomatosis, sarcoidosis, and mitral stenosis. Serial biopsies demonstrate the effectiveness of streptomycin treatment.—*La ponction biopsie du foie dans les tuberculoses miliaires, M. Janbon, P. Cazal, & L. Bertrand, Presse méd., September 28, 1949, 57: 854.—(V. Leites)*

Pneumoperitoneum.—One hundred patients were treated with pneumoperitoneum during a period of two years. The duration of treatment was six to twenty-four months, averaging eleven months. In 20 of the 100 patients, the sputum became negative for acid-fast bacilli; in 17 it remained negative after two years. Marked clinical improvement occurred in 52 patients during the first two to three months of treatment. The worst results were obtained in patients with disseminated disease. When basal emphysema was associated with tuberculosis, pneumoperitoneum improved

the respiratory function by raising the diaphragm. Puncture of the intestines occurred in 2 patients, mediastinal emphysema in 3. Both complications regressed spontaneously. The following are considered indications for pneumoperitoneum: (*1*) bilateral infiltrations which preclude pneumothorax because of their extent and impairment of respiratory function; (*2*) bilateral involvement where pneumoperitoneum can be used preparatory to unilateral pneumothorax; (*3*) disease in the middle or lower lobes which prevents the use of thoracoplasty for other lesions; (*4*) infiltration in older patients, especially if associated with emphysema; (*5*) bronchogenic aspiration after hemoptysis; (*6*) acute exudative exacerbations occurring before phrenic paralysis; (*7*) cavitary disease associated with endobronchial tuberculosis, provided the cavity is less than 5 or 6 cm. in diameter and is situated in the lower or central parts of the lung; (*8*) exacerbations after childbirth, unilateral lesions being subsequently treated with pneumothorax; (*9*) pleural effusion with minimal lung involvement so that the establishment of pneumothorax is not contemplated. The duration of pneumoperitoneum depends on extent and character of the disease but should always exceed two years. Contraindications to pneumoperitoneum are far advanced disease with toxic manifestations, marked cardiac decompensation (especially cor pulmonale), pregnancy, and tumors in the abdominal cavity.—*Pneumoperitoneum as a method of collapse therapy* (*Russian*), *A. J. Zcigelnik & V. J. Shapiro, Probl. tuberk., May–June, 1949, 3: 23.*—(*V. Leites*)

Roentgen Therapy of Adenitis.—Cervical tuberculous lymphadenitis in 37 patients was treated by concentrated roentgen therapy and conservative surgical procedures. Roentgen treatment was given daily at 200 r for four or five days to one field large enough to include the obviously diseased nodes and the regional group. Complete regression of the diseased nodes and healing of sinuses occurred in 84 per cent of the patients; in about 20 per cent of these a second course of irradiation was necessary two to three months later to achieve this result. If the patient did not respond properly to the second course, no further irradiation was given. When response was satisfactory it was also early. The duration of the enlargement prior to irradiation and the presence of draining sinuses had no effect on the results. This concentrated roentgen therapy over four to five days is convenient, and the results are comparable to the best in literature. For the present, radiation therapy must be regarded as superior to streptomycin in the management of cervical tuberculous adenopathy. Streptomycin might best be reserved for use in the 15 to 20 per cent of all cases where irradiation does not cause complete regression.—*Concentrated roentgen therapy of cervical tuberculous lymphadenitis, I. Lampe, C. P. Chrest & D. A. Koch, Am. J. M. Sc., June, 1949, 217: 632.*—(*W. J. Steininger*)

Vitamin D_2 for Skin Tuberculosis.—Of 68 patients treated with massive doses of vitamin D_2 (calciferol), 63 had lupus, 3 had scrophuloderma, one had papulonecrotic tuberculids and erythema Bazin, and one had erythema Bazin. Among the 63 patients with lupus, 40 had ulcerative lesions, mostly on the face; 17 had disseminated lupus. The duration of illness ranged from one to twenty-five years. Treatment of 37 patients consisted of intramuscular injections of 600,000 units of vitamin D_2 in oil every three days for two to four weeks, followed by the same dose once weekly. Another 32 patients were given 100,000 units in alcoholic solution once daily. The duration of treatment ranged from two to eight months. Clinical cure was achieved in 21 patients; 29 were considerably improved, 15 were moderately improved, and 4 remained unimproved. Clinical cure was observed in 54 per cent of the patients treated at least four months and in 72 per cent of those treated five months and longer. Focal reactions with increasing redness, exudation, and sometimes increased ulceration were observed at the beginning of treatment in 14 patients; 11 showed perifocal reactions around their

pulmonary lesions; 8 developed enlargement of the regional lymph nodes. In connection with these findings, increased tuberculin sensitivity was noted in 29 patients. Vitamin D_2 also had toxic effects on the liver and kidneys, in some cases necessitating lowering of the dosage. Biopsy specimens during treatment showed early proliferation of connective tissue elements and regression of the cellular components of the tuberculous lesions.—*Treatment of skin tuberculosis with Vitamin D (Russian), M. J. Menj & M. G. Podgaezkaja, Probl. Tuberk., March–April 1949, 2: 24.*—(*V. Leites*)

Avian Tuberculosis.—An acid-fast infection resembling avian tuberculosis in a wild, adult, male sparrow-hawk produced lesions in the liver, lungs, and two major joints with their associated ligamentous and muscular structures. Grossly and histologically the appearance was consistent with a tuberculous infection. Acid-fast organisms having the morphologic characteristics of *Mycobacterium tuberculosis* were found. The findings were in keeping with an initial infection by way of the alimentary tract and later spread by the blood and lymphatics as well as by direct extension.—*Tuberculosis in a wild sparrow-hawk (Accipiter nisus nisus), J. M. Harrison, J. Path. & Bact., October, 1948, 60: 583.*—(*H. J. Henderson*)

CLINICAL STUDIES, NONTUBERCULOUS DISEASES

Sarcoidosis.—From the files of the Army Institute of Pathology, 300 consecutive cases with lesions diagnosed as sarcoidosis were analyzed. All lesions conformed to the histologic criteria for the disease as adopted by the Conference on Sarcoid, National Research Council, in February 1948. (*1*) Clinical findings: The greatest incidence was in the 20- to 24-year age group and the disease occurred seventeen times as often in Negro as in white soldiers. Most of the cases came from Army hospitals, 271 being in men from 18 to 39 years of age. This indicates a wider prevalence of sarcoidosis, especially among males, than has theretofore been demonstrated. Among the 258 patients who presented symptoms on admission, the most common complaints were enlarged lymph nodes (86 per cent), cough (20 per cent), weight loss (19 per cent), skin manifestations (16 per cent), low-grade fever (15 per cent), malaise (10 per cent), and weakness (10 per cent). In the entire group of 300 patients, the organs most frequently affected were lymph nodes (83 per cent), skin (21 per cent), bone (17 per cent), eyes and liver (each 9 per cent), and spleen (8 per cent). Other organs were each involved in less than 3 per cent of the cases. Over two-thirds of the patients were admitted to hospitals within six months after they first noticed symptoms. Roentgenologic examinations showed mediastinal widening in 76 per cent of the patients examined; small bone involvement was seen in 36 per cent of the patients who had roentgenologic examinations of the extremities. Tuberculin skin tests were negative in 97 per cent of 88 patients. The sedimentation rate was elevated in 62 per cent of 52 patients. Hyperproteinemia of 8 Gm. or more per 100 ml. of blood was present in one-third of 40 patients. Both albumin and globulin fractions were recorded in 31 instances, and in 19 (over 60 per cent) the globulin was 3.0 Gm. or higher for each 100 ml. of blood. (*2*) Follow-up: Seventy-five per cent of the patients answering a follow-up questionnaire reported the recurrence of symptoms consistent with sarcoidosis; 94 per cent knew of no other similar cases occurring at the same time and place as theirs. The other 4 (6 per cent) each reported one other similar illness, but this is questionable. The majority suggested no cause; most of the remainder attributed their trouble to exposure, overexertion, or upper respiratory infections. (*3*) Autopsied cases: Twenty-two autopsied cases of sarcoidosis are added to 62 collected from the literature. Sarcoidosis was unsuspected until autopsy in 14 of the 22, and in only 3 was death unequivocally due to sarcoidosis. Three of the 22 patients had associated sarcoidosis and tuberculosis, and 2 others showed abacterial, caseating lesions of the adrenal glands with

clinical Addison's disease. Two surviving patients from whom tissue was removed at operation also showed associated tuberculosis. (4) Histopathology: In lymph nodes, proliferation of the sinusoidal reticulum almost invariably preceded the development of the tubercles of sarcoidosis. The miliary granuloma of sarcoidosis was characteristic and in its adult form appeared as a discrete, sharply delimited lesion, composed of concentrically arranged, variably elongated, epithelioid cells. Since all the granulomas in one organ were usually at the same stage of development, a peculiar monotony of pattern resulted. The lesions of sarcoidosis healed by fibrosis. Giant cells of both Langhans' and foreign-body type were common. Giant cell inclusions occurred in 6 per cent of the material, Schaumann bodies in 4 per cent, and spiculated bodies ("asteroids") in 2 per cent. None of these was specific for sarcoidosis. The latter were always found in giant cells; the former often were extracellular, looking as though they had been extruded from an adjacent giant cell. "Fibrinoid" necrosis, usually distinguishable from the caseation of tuberculosis, occurred in 35 per cent of this material.—*Sarcoidosis. A clinicopathologic review of three hundred cases, including twenty-two autopsies, W. Ricker & M. Clark, Am. J. Clin. Path., August, 1949, 19: 725.—(J. S. Woolley)*

Tuberculo-complement-fixation in Sarcoidosis.—In tuberculous animals, antibodies to *M. tuberculosis* have been demonstrated after skin sensitivity to tuberculin has waned. If sarcoidosis were a form of tuberculosis, it might be anticipated that such antibodies would be found in patients with sarcoidosis even in the absence of tuberculin sensitivity. Complement-fixing antibodies to *M. tuberculosis*, therefore, were sought for in the sera of 22 patients with sarcoidosis proved by biopsy. The chest roentgenogram of each patient was free of characteristic tuberculous lesions; in the majority there were lesions typical of sarcoidosis. The superficial lymph nodes were enlarged in 14 patients, the mediastinal nodes in 13, and the abdominal nodes in 2. The lungs were involved in 13 instances, the eyes in 10, the skin in 6, the parotid glands in 5, the mucosae in 2, and the bones and spleen in one each. Biopsy stains for tubercle bacilli were always negative; guinea pigs were inoculated with biopsy material from 16 patients, but tuberculosis never developed. Material from a gastric lavage from a 19-year-old Negro youth with typical uveoparotid fever produced tuberculosis in a guinea pig. None of the 16 patients tested acted to 0.01 mg. of Old Tuberculin; 2, tested with 0.1 mg., gave positive reactions; and 2 others, who were tested with 1.0 mg., were negative. In every case where the tuberculin test was positive, biopsy material was negative on guinea pig inoculation. The ages ranged from 19 to 65 years with a mean of 28; there were equal numbers of men and women. Thirteen patients (59 per cent) were Negroes. A group of 26 patients with active tuberculosis was used for comparison. Only one of these had a negative tuberculin test and he developed a fatal meningitis. Further comparison was made with healthy medical students who reacted positively to the first test dose of PPD. Complement-fixation reactions were performed on sera from the 22 patients with sarcoidosis, using a variety of antigens prepared from *M. tuberculosis* H37. Positive serologic reactions were obtained in 5 (27.3 per cent) of the cases. In contrast, 16 (61.7 per cent) of 26 patients with active tuberculosis gave positive serologic reactions and the average titer was higher in the sera of this group than in that of patients with sarcoidosis. Sera from 10 (33.3 per cent) of 30 healthy students with positive tuberculin tests gave positive complement-fixation reactions with a range of titers similar to that in the sera from patients with sarcoidosis. The positive complement-fixation reactions may be due to past tuberculous infection unrelated to sarcoidosis. Unless patients with sarcoidosis have an impaired ability to develop complement-fixing antibodies to tubercle bacilli, these results indicate that this disease is not tuberculous.—*A comparison of sarcoidosis and tuberculosis with respect to complement*

fixation with antigen derived from the tubercle bacillus, W. H. Arnes & S. Raffel, Bull. Johns Hopkins Hosp., September, 1949, 85: 204.—(J. S. Woolley)

Pulmonary Schistosomiasis.—Of 103 cases of schistosomiasis, roentgenograms of 49 revealed abnormal pulmonary findings. Lesions were almost entirely confined to the pulmonary vascular system and could be seen as nodulation, thickening, or tortuosity of the vascular network. With greater chronicity, enlargement of the hilar root vessels and of the right ventricle of the heart occurred. Radiologic differential diagnosis included miliary tuberculosis, periarteritis nodosa, Loeffler's syndrome, passive congestion, pneumonia, and congenital cardiac lesions. Treatment of the schistosomiasis with tartar emetic often produced an increase in the patchy, confluent pulmonary densities, compatible with bronchopneumonia, and an increase in the size of the cardiac shadow by as much as 1 cm. These reactions gradually subsided and the lungs became clear while the heart regained its previous size.—*The radiological features of chronic pulmonary schistosomiasis, H. Erfan & A. A. Deeb, Brit. J. Radiol., November, 1949, 22: 638.—(B. Hyde)*

Chronic Bronchitis.—Two hundred cases of chronic bronchial inflammation are divided in four groups; (*1*) 92 cases of simple bronchial catarrh; (*2*) 31 cases of simple purulent bronchitis; (*3*) 21 cases of purulent bronchitis with distension; (*4*) 32 cases of bronchiectasis. All these patients were subjected to thorough examination of the nose and throat. Pathology of the upper air passages was found in 80 per cent. In the group with simple bronchial catarrh, 25 per cent of the patients had sinusitis, 30 per cent had significant deviation of the nasal septum, 4 per cent had chronic tonsillitis; 12 patients had enlarged tonsils and adenoids and 5 patients had rhinopharyngitis. There was no nasal or pharyngeal pathology in 25 per cent of this group. In the group with purulent bronchitis, 68 per cent of patients had sinusitis, 17 per cent had a deviated septum; 2 patients had inflammation of the Eustachian tube, one had ozena. In all, 90 per cent of the patients had pathologic findings. In the group with purulent bronchitis and distension, bronchoscopy showed an inflamed mucosa and distended bronchial orifices. Lipiodol studies revealed a "leafless-tree" appearance of the bronchi. Sinusitis was found in all of these patients, deviated septum in 10 per cent. Of the patients with bronchiectasis, 87 per cent had sinusitis; 5 per cent had no nasal or pharyngeal pathology.—*La frequence méconnue des lesions des voies aeriennes supérieures dans les affections catarrhales ou suppurées des bronches avec ou sans dilatation, R. Kourilsky, M. Fourestier, Y. le Boucher, M. Boiteux, & E. de Saint-Germain, Semaine d. hôp. Paris, November 6, 1949, 3409.—(V. Leites)*

Friedländer Pneumonia. — Friedländer pneumonia is relatively infrequent but it is nevertheless very important because of its severity, the high incidence of complications, and the markedly elevated mortality rate. Early recognition of the disease, especially by roentgenographic diagnosis, and the prompt institution of therapy are of utmost importance. The roentgenographic findings are variable and classified into 3 groups: (*1*) Massive lobar consolidation in which a single lobe or an entire lung may be involved. In some instances the affected lobe seems to bulge, fluid or "drowned lung" being suspected. (*2*) Lobular consolidation in which there is a patchy, irregular density which later develops into confluent consolidation scattered through one or more lobes. (*3*) Chronic changes characterized by pulmonary suppuration and lung abscess formation. The lung abscesses may form relatively early in the course of Friedländer pneumonia. It is in cases of massive lobar consolidation that the roentgenologist may be of greatest aid to the clinician. Massive consolidation involving one or more lobes should immediately suggest the possibility of Friedländer pneumonia.—*The clinical and roentgen manifestations of pneumonia due to bacillus*

mucosus capsulatus (primary Friedländer pneumonia), M. Ritvo & F. Martin, Am. J. Roentgenol., August, 1949, 62: 211.—(T. H. Noehren)

Loeffler's Syndrome.—A case of pneumonia associated with pericardial effusion and venous thrombosis is reported. The role of hypersensitivity in this patient's illness is suggested by a history of bronchial asthma and the discovery of eosinophilia in the blood, sputum, and pericardial fluid. The patient had 3 illnesses which were, in all probability, recurrent episodes of Loeffler's pneumonia. Following the first 2 episodes complete resolution occurred and no residual pulmonary damage was evident clinically or roentgenographically. In the third episode, the onset was similar and for a time the changes were limited to the lungs. Then serous membrane involvement became evident in the pericardium and pleura. The pericardial effusion subsided but the subsequent course was characterized by continued difficulty in breathing without asthmatic episodes suggesting that constrictive pericarditis had developed. The appearance of thrombophlebitis without recognized cause may have been due to vascular damage from the same allergic stimulus. The course of this patient is similar to that of the 16 reported by Harkovy, 15 of whom were suffering from typical bronchial asthma and had pulmonary lesions with reactions of serous membranes, including pleura, pericardium, and peritoneum.—*Loeffler's syndrome with associated eosinophilic polyserositis, H. A. Dickie & E. Grimm, Am. J. Med., November, 1949, 7: 690.—(T. H. Noehren)*

Bronchopulmonary Infections.—In 5 patients there were segmental pulmonary consolidations and bronchial inflammation limited to the involved segments. The etiology was most often a descending infection from the nasopharynx. Various pathogenic organisms were found, but gram-negative cocci and rods were predominant. Two of the 5 cases had small hemoptyses. The segmental consolidation showed no roentgenological signs of retraction indicating that there was no bronchial occlusion despite the inflammatory change seen bronchoscopically. The clinical course was protracted, but regression took place after a period of several months. All the patients recovered completely. The segmental pulmonary involvement apparently was not secondary to the bronchial disease but both were due to the same descending infection. The condition did not respond well to parenteral or local administration of antibiotics. A superimposed vascular factor may explain the special features of the syndrome.—*Les infections broncho-pulmonaires ségmentaires, R. Kourilsky, S. Kourilsky, E. Decroix, & C. Lifshitz, Semaine d. hôp. Paris, November 6, 1949: 3395.—(V. Leites)*

Arterial Air Embolism.—Sources of air in arterial air embolism are: (*1*) Intrinsic from (*a*) air in the alveoli; (*b*) air in pulmonary cavities; or (*c*) air in the pleural cavity from pneumothorax. (*2*) Extrinsic from (*a*) injected air; (*b*) air aspirated from the tubing of the pneumothorax apparatus; or (*c*) air aspirated into pulmonary veins during open-chest procedures. The symptoms are dizziness, fainting, pain in the head, loss of consciousness, convulsion, various localized neurologic signs such as hemiplegia, hemiparesis, hemianesthesia, monoplegia, hemianopsia, nystagmus, strabismus, and blindness. Cyanosis, respiratory disturbances, and peripheral vascular collapse also occur. The pathognomonic signs are detection of air in the retinal vessels by ophthalmoscopic examination; Liebermeister's sign, sharply defined areas of pallor in the tongue; marbling of the skin, air bubbles in the blood escaping from an incision made in the skin over the most superior portion of the body; and roentgenologic demonstration of air in the cerebral vessels. On the basis of experiments performed in dogs the following conclusions are made: "... air introduced into the coronary circulation, either directly or by injection into the pulmonary vein or left auricle, produces ischemia of the myocardium in areas supplied by involved vessels.

The ischemia is demonstrable grossly and by electrocardiographic examination. It may be very temporary with speedy recovery, or it may persist even after the apparent complete disappearance of the gas bubbles. Death from ventricular fibrillation may result within a very short time after the injection of the air. The distribution of air within the arterial circulation is determined by the principle of air buoyancy. This principle may be made use of in the therapy of arterial air embolism."—*Arterial air embolism, T. M. Durant, M. J. Oppenheimer, M. R. Webster, & Joan Long, Am. Heart J., October, 1949, 38: 481.*—(*G. C. Leiner*)

Pneumoconiosis in Graphite Workers.—Exposure to graphite dust is an occupational hazard in several trades. Several cases occurred among men employed in a factory producing "black lead", a mixture used in the cleaning of fireplaces. Analysis of the finished "black lead" showed carbon, 75 per cent; total silica, 8.7 per cent; silica, 0.1 per cent. In no instance had any of the patients been employed previously in any other dusty occupation. The signs and symptoms of pneumoconiosis were sometimes slight and nonspecific; the diagnosis could be established only by radiologic examination. Repeated sputum examinations failed to reveal acid-fast bacilli. The onset of the radiologic lesions or their extent seemed to bear no relationship to the duration of employment in the graphite factory but there appeared rather to be variation in the individual susceptibility. Several patients expectorated large amounts of black sputum over varying periods, and others had bluish deposits under the skin of the hands and forearms. These stains resembled tattoo marks and had probably been produced by small amounts of graphite being forced into the subcutaneous tissues. There were no local irritant effects from this graphite beneath the skin. The chest roentgenograms were not pathognomonic of "graphite lung". Marked striation, fibrosis, nodulation, large opaque patches, and combinations of these changes occurred unilaterally or bilaterally. A miliary "snow storm" pattern and sharply defined masses which varied in size and opacity deserve particular mention. One of the 2 cases reported in detail died and at autopsy the numerous dense masses seen in the roentgenograms were found to be cysts completely filled with black fluid. No communication was demonstrable between cysts and bronchi.—*Pneumoconiosis in graphite workers, L. Dunner & D. J. T. Bagnall, Brit. J. Radiol., October, 1949, 22: 573.*—(*B. Hyde*)

Pneumoconiosis Caused by Graphite.—Graphite is a form of carbon which is very soft. It occurs naturally, being almost universally distributed in nature and is also produced synthetically, particularly in the Niagara Falls area. In neither instance does it appear in pure form. Impurities consist of silica and iron oxides which are often as high as 35 per cent. It has very wide industrial use, and dustiness characterizes most of its applications. If it had harmful effects, these certainly would be well known. Such is not the case. Long exposure may result in benign pneumoconiosis; no proved disability results from it. Silicosis may, however, result from the impurities. Roentgenographically, characteristic snowstorm opacities are seen. These may represent the graphite alone and not a tissue reaction. All three forms, crystalline, flake, and amorphous, are equally innocuous.—*Graphite (plumbago, black lead), as a source of dusty lung disease, C. P. McCord, Indust. Med. & Surg., November, 1949, 18: 483.*—(*A. G. Cohen*)

Chest Pathology in Infectious Mononucleosis.—Changes in the chest roentgenogram in infectious mononucleosis are infrequent. On the basis of published pathologic studies, it is suggested that pulmonary changes, when present, are due to engorgement of the hilar lymph nodes and infiltration of the interstitial tissue of the lung by atypical lymphocytes and monocytes. In 7 cases of clinically proved infectious mononucleosis, roentgenographic examination showed the following: (*1*) enlargement of the mediastinal

lymph nodes in 2 cases; (*2*) both enlargement of the mediastinal lymph nodes and pulmonary parenchymal changes in 3 cases; (*3*) parenchymal changes only in 2 cases.—*Infectious mononucleosis with special reference to roentgenologic manifestations, J. J. McCort, Am. J. Roentgenol., November, 1949, 62: 645.*—(*T. H. Noehren*)

Ductus Arteriosus Aneurysm.—Aneurysm of the ductus arteriosus is a rare condition, with 31 cases now reported but it is of importance to the roentgenologist and the thoracic surgeon as a type of mediastinal tumor. The attempted removal of such a tumor could result in uncontrollable hemorrhage. The peculiar location of such a tumor is its most significant feature: (*1*) In posteroanterior and anteroposterior views the aneurysm extends to the left and slightly above the pulmonary conus. (*2*) In the right anterior oblique view the aneurysm is projected as a tumor mass to the left and superior to the pulmonary conus, lying between the latter and the aortic arch. (*3*) In the left anterior oblique view the clear shadow of the "aortic window" is obliterated by the tumor produced by the ductus aneurysm. (*4*) The lateral view in the author's case shows the aneurysm to be anterior to the trachea and to extend farther anteriorly than either the pulmonary conus or the aorta. This is the only feature of the present case not in accordance with the diagnostic criteria laid down by Mackler and Graham who found that the aneurysm was posterior to the trachea in their 3 cases. (*5*) The ductus aneurysm would produce a tumor shadow in the anterior mediastinum and in the lower portion of the superior mediastinum. Visualization of the aneurysm with diodrast using the technique of Robb and Steinberg should establish the diagnosis. (Author's summary)—*A case of aneurysm of the ductus arteriosus with postmortem roentgenologic study after instillation of barium paste, J. H. Kneidel, Am. J. Roentgenol., August, 1949, 62: 223.*—(*T. H. Noehren*)

Vascular Tracheal Constriction.—Severe stridor and dysphagia were noted by patients in whom there is a right aortic arch with the thoracic aorta descending on the right side. In these persons a ligamentum arteriosum arises from the pulmonary artery, passes to the left of the trachea and esophagus, and then circles behind the esophagus to the thoracic aorta producing a constrictive ring. On roentgenographic examination these patients showed the aortica vascular mass to the right of the midline. An indentation was seen on the right side of the barium-filled esophagus in the anterior-posterior projection and in the left anterior-oblique view. There was also a small defect on the posterior aspect of the esophagus produced by the ligamentum arteriosum as it passes behind the esophagus from the left side. The trachea showed a shallow extrinsic pressure defect on the right side.—*Tracheo-esophageal constriction produced by right aortic arch and left ligamentum arteriosum, E. B. D. Neuhauser, Am. J. Roentgenol., October, 1949, 62: 493.*—(*T. H. Noehren*)

Cavernous Hemangioma in the Lung.—Pulmonary varices of a tumorous type are extremely uncommon and practically all of the cases have been reported in the last decade. Another case of cavernous hemangioma of the lung occurred in a 28-year-old woman. Recovery followed a right lower lobectomy performed because the lesion was believed to be a tuberculoma. The pathogenesis of the tumor is not clear but a congenital origin seems most likely.—*Varizes pulmonares, G. Botelho & D. Fleury de Amorim, Rev. brasil. de tuberc., March–April, 1949, 17: 199.*—(*A. A. Moll*)

Pulmonary Adenomatosis.—What is considered to be the first case of pulmonary adenomatosis from Canada is reported. The patient had extensive pulmonary lesions and was carrying on a normal life when the lesion was found by mass community roentgenologic survey. The sputum pathology was positive.—*Pulmonary adenomatosis with a new laboratory*

finding, W. H. Hatfield & J. E. Hill, Am. J. Roentgenol., October, 1949, 62: 525.—(T. H. Noehren)

Sputum Cytology in Bronchial Carcinoma.—Cytologic examination of sputum and bronchial secretions for cancer has been done in the authors' laboratory for nearly three years. For the test five smears from each specimen were fixed, while wet, in the alcohol-ether mixture of Papanicolaou, and stained with hematoxylin and eosin. The number of sputum specimens averaged approximately two from each patient; a single bronchial specimen usually was available. In a group of 588 recent cases of thoracic disease, routine cytological examination of sputum or bronchial secretions was performed. Bronchogenic carcinoma was proved by biopsy or exploration, or strongly suspected in 147 patients. In 100 of these 147 patients, the presence of cancer was detected by the cytologic examination. Hence the procedure was 68 per cent accurate and there were only one or two false positive results.—*Cytologic diagnosis of bronchogenic carcinoma, L. B. Woolner & J. R. McDonald, Am. J. Clin. Path., August, 1949, 19: 765. (J. S. Woolley)*

Pleural Adhesions.—A study of 6,986 autopsy reports at the Institute for Legal Medicine in Milan, Italy, showed that pleural adhesions were present in about 50 per cent of all the cases examined. The autopsies were made on 5,087 apparently healthy individuals (3,807 men and 1,208 women) and on 1,908 persons (1,462 men and 446 women) who had died as the result of disease. Adhesions were found in 45 per cent of the women and in 55 per cent of the men. In the diseased group alone, the incidence was 55 per cent in women and 62 per cent in men. The percentage of individuals found with adhesions increased with age, but bilateral involvement was comparatively more frequent in the younger age groups. The presence of adhesions which can be demonstrated only post mortem in so many persons leading a normal life suggests that the lesions are, with few exceptions, of little importance.—*Menomazione dell'integrità fisica dell'individuo dipendente dalle aderenze pleuriche, M. Alfero, Gior. ital. tubere., January-February, 1949, 3: 58.—(A. Mochi)*

Differentiation of Mediastinal Tumor and Aneurysm.—Angiocardiography is advocated as a simple and effective method for differentiating aneurysms from other space-occupying lesions of the thorax. The authors have employed a stereo cassette changer and have routinely used only two exposures per injection. Errors in diagnosis have arisen because aneurysms may not show pulsation due to clotting, while tumors may show transmitted pulsation; these difficulties are readily resolved by angiocardiography. Four patients are presented, all of whom have a positive Wassermann reaction. The first was shown to have a tumor, and the other 3 aneurysms. One patient had an aneurysm of the left subclavian artery in addition to an aneurysm of the aortic arch. The former did not opacify due to the small size of the orifice communicating with the aorta. (Author's summary).—*The differentiation of mediastinal tumour and aneurysm: Value of angiocardiography, I. Steinberg & C. T. Dotter, Brit. J. Radiol., October, 1949, 22: 567.—(B. Hyde)*

Bronchial Peristalsis.—Assumptions that peristaltic motion occurs within the bronchi have been based on the roentgenologic observation of the breaking up of opaque oil columns and the slow centripetal motion of oil particles. It is suggested that the droplets of oil, by means of their viscosity and surface tension may elevate an abnormally large mucosal fold making the lumen appear narrower. The slow centripetal motion of these droplets should be interpreted as caused by the action of the ciliated epithelium. Indeed constricting rings are never seen in bronchi completely lined with oil and otherwise filled with air. The usual muscular arrangement for peristalsis also is lacking in that there are no longitudinal muscular systems in the bronchi.—*Bronchial peristalsis, F. G. Fleischner, Am.*

J. Roentgenol., July, 1949, 62: 65.—(T. H. Noehren)

Angiocardiography.—Aberrant insertion of the pulmonic veins associated with an interarterial septal defect was diagnosed by angiocardiography with the serial-exposure technique. These anomalies can be easily recognized once the observer is acquainted with their existence and roentgenographic appearance. Aside from angiocardiography, right vena caval catheterization should prove helpful in confirming the diagnosis. A greater oxygen saturation of blood from the right atrium and ventricle than from the vena cava superior or inferior might be due to the aberrant vein or to the interatrial septal defect. The aberrant insertion of one pulmonic vein is compatible with long life; the abnormal insertion of more than one pulmonic vein into the main tributaries of the right atrium is rarely compatible with life beyond infancy. The diagnosis of congenital pulmonic arteriovenous fistula can also be made by angiocardiography. Apparently aberrant and accessory pulmonary arteries and veins are frequent.—*The roentgenographic and angiocardiographic aspects of (1) aberrant insertion of pulmonary veins associated with interatrial septal defect and (2) congenital arteriovenous aneurysm of the lung, A. Grishman, M. H. Poppel, R. S. Simpson, & M. L. Sussman, Am. J. Roentgenol., October, 1949, 62: 500.—(T. H. Noehren)*

ANTIMICROBIAL THERAPY

TB 1 and TB 4 for Tuberculosis.—At the Taunusheim Sanatorium, 50 patients with a diagnosis of active pulmonary tuberculosis characterized by cavitary components were treated with 4-acetylaminobenzaldehyde thiosemicarbazone (TB 1) or a sulfathiadiazole (TB 4). In more than half of the patients extrapulmonary tuberculosis was also present. TB 4 was administered by intravenous or intrapleural injections or by inhalation. Beneficial effects such as defervescence, decrease in cough, and amount of sputum were noted. Roentgenographically, the cavities remained unchanged but pericavitary infiltration was favorably influenced. The dosage had to be individualized for optimal results. In some patients 25 mg. sufficed for the acute exudative component whereas in others 100 mg. was insufficient for the chronic type of proliferative disease. The general condition of the patient and roentgenographic and laboratory findings influenced the amount of TB 1 to be used. While the pulmonary pathology did not show impressive changes, intestinal tuberculosis was favorably influenced. Oral administration of TB 1 was continued over a 200- to 300-day period. Better results were obtained by using TB 1 with sulfathiazole (TB 1/E). Tuberculosis of the larynx was favorably influenced by daily local applications of 200 mg. of the TB 1 powder. The earlier use of the water-soluble 10 per cent TB 4 solution was replaced by inhalation of water-insoluble TB 1/698. Toxic manifestations with TB 1 occurred in a few patients and consisted of icterus, conjunctivitis, photophobia, and dermatitis.—*Zur Chemotherapie kavernöser Lungentuberkulose und ihrer spezifischen Komplikationen mit TB 1 und TB 4, W. Viets & H. Scholtze, Tuberkulosearzt, 1949, No. 9: 508.—(E. Dunner.)*

TB 1/E for Tuberculous Empyema.—At the Heilstätte Sanatorium near Warstein, 32 patients with tuberculous empyema of the pleural cavity were treated by frequent aspirations, irrigation with physiological saline, and instillation of a suspension of 4-acetylaminobenzaldehyde thiosemicarbazone and sulfathiazole in equal parts (TB 1/E). The amount of TB 1/E used varied from 500 to 1,000 mg. in 20 cc. of physiologic saline solution depending upon the severity of the infection. General improvement, defervescence, and decrease in sedimentation rate were noted frequently. It appears important to use prolonged treatment in order to prevent an exacerbation of symptoms. After an average treatment period of five weeks, during which time weekly instillations of TB 1/E were employed, beneficial results were obtained in all except 3 patients. Oral medication with the thiosemicarbazone was not effective in the treatment of tubercu-

lous empyema.—*Chemotherapie tuberkulöser Pleuraempyema mit TB 1-698, F. Koch, Beitr. z. Klin. Tuberk., 1949, No. 2: 235.*—(*E. Dunner.*)

TB 1 for Tuberculous Laryngitis.—Between March, 1947 and September, 1948, 41 patients with active tuberculosis of the larynx were treated in the Schleswig-Holstein Sanatorium in Mölln with 25 to 50 mg. of 4-acetylaminobenzaldehyde thiosemicarbazone (TB 1) daily for varying periods up to twenty weeks. Pain and dysphagia disappeared in four to five days permitting the patients to eat better and gain weight. The rate of improvement depended upon the degree of involvement. In mild cases the first signs of healing were shown in five to fourteen days and the lesions appeared healed in approximately three to four weeks after a total dose of 7 Gm. In moderately severe cases healing was first evident in two to three weeks and appeared complete in five to eight weeks after a total dose of 35 to 40 Gm. Toxic manifestations which caused discontinuation of therapy were agranulocytosis and exanthemata. Treatment was interrupted in some cases due to emesis, loss of weight, severe conjunctivitis, excessive urobilinogen, anemia, urticaria, stomatitis, and vulvitus. Of the 41 cases treated, 32 (78 per cent) were reported as healed.—*Erfahrungen in der Behandlung der Kehlkopftuberkulose mit TB 1-698 E von Domagk, E. Stutz, Beitr. z. Klin. Tuberk., 1949, No. 1: 57.*—(*E. Dunner.*)

Agranulocytosis with Thiosemicarbazone.—Two cases of agranulocytosis occurred during thiosemicarbazone therapy for active pulmonary tuberculosis. In the first patient it appeared on the thirtieth day of treatment and in the second patient on the thirty-sixth day. Both cases received 100 mg. of 4-acetylaminobenzaldehyde thiosemicarbazone (TB 1) daily. A leukopenia of 800 and 1,200, respectively, developed. Symptomatology included a sudden elevation of temperature, malaise, headache, and inflammatory changes in the oropharyngeal region. Necrotic lesions did not occur. Therapeutically, penicillin and blood transfusions were prescribed successfully.—*2 Fälle von schwerer Agranulocytose als Nebenerscheinungen bei der Behandlung der Tuberkulose mit TB 1-698, W. Merkel, Tuberkulosearzt, 1949, No. 9: 518.*—(*E. Dunner.*)

Anemia Due to Thiosemicarbazone.—Of 89 patients who received a total of more than 10 Gm. of 4-acetylaminobenzaldehyde thiosemicarbazone (TB 1) for the treatment of tuberculosis, there was a reduction in hemoglobin of more than 10 per cent in 36 cases. The anemia appeared as early as the third to fourth week of treatment in some, and in others, it appeared as late as the seventeenth to eighteenth week. It was noted more frequently and earlier in the exudative type of disease than in the productive or mixed types. In the exudative cases, dosage also appeared to be a factor with anemia appearing in 11 patients of 18 who received over 150 mg. of thiosemicarbazone daily as compared with 4 cases of 14 who received a dose of less than 150 mg. per day.—*Hämatologische Beobachtungen bei Behandlung mit TB 1-698, W. Pribilla and H. Otte, Tuberkulosearzt, 1949, No. 11: 633.*—(*E. Dunner.*)

Streptomycin for Tuberculous Meningitis.—Of 100 cases of tuberculous meningitis admitted to the Laennec Hospital in Paris during 1947 and 1948, 98 were adults, 73 of the patients being 15 to 25 years of age and 25 being older. At the end of the observation period, 22 patients were alive and well. Of these, 17 had been followed for about two years and the remaining 5 for thirteen to eighteen months. In all, 78 of the 100 patients died, 18 per cent in the first five days and 67 per cent in the first six months. The risk of mortality was negligible after the ninth month. Of the 22 patients who were discharged, none had neurologic sequelae or psychic defects. The diagnosis of tuberculous meningitis was made in 78 cases by direct examination of the cerebrospinal fluid and in 16 cases by culture or animal inoculation. In 18 patients there was evidence of a primary infection within the preceding six months. In no case had BCG been admin-

istered. Of the 100 patients, 40 had associated miliary tuberculosis. Streptomycin was given in a series of 10 to 15 intrathecal injections with a total dose of 1.0 to 1.5 Gm. Another series was administered if the effect of the first on clinical symptoms and spinal fluid was deemed unsatisfactory. Intramuscular injections of 1.5 to 2.0 Gm. of streptomycin were given daily. A correlation was found between the risk of relapse and duration of treatment. The risk was 100 per cent in cases treated for less than three months, 50 per cent in cases treated five to seven months, and 14 per cent in cases treated seven to nine months. No relapse was noted in cases treated over nine months. In the 17 "cured" cases without relapse, the duration of treatment was from 121 to 330 days. Parenteral administration of streptomycin should not be less than seven months. Relapses were immediately and energetically re-treated. Pathological findings in the spinal fluid usually preceded the appearance of the clinical symptoms of relapse. Of 15 relapses, 5 (33 per cent) responded favorably to re-treatment. In 11 of these, the bacilli had remained sensitive to streptomycin. The prognosis in relapse was not found more grave than that in the first attack.—*La meningite tuberculeuse de l'adulte traitée par la streptomycine. Deux années d'experience, E. Bernard, B. Kreis, A. Lotte, P. Chiche, & P. V. Paley, Semaine d. hôp. Paris, June 30, 1949: 2054.—(V. Leites)*

Streptomycin for Tuberculous Sinuses.—Eleven patients with 28 tuberculous sinuses were treated with streptomycin. The sinuses arose from bone and joints in 5, from thoracoplasty wounds in 3, after nephrectomy in 2, and after Monaldi drainage in one. The dose of streptomycin was 1.0 to 1.5 Gm. daily. There was good improvement in the patients' general condition with a disappearance of toxemia and an increase in appetite. Locally, the discharge from the sinus became less in three to four weeks. Eventually the sinus healed; this required three months in 75 per cent and more than three months in the remainder. There were no recurrences. The response was particularly dramatic when the sinuses arose from bones and joints. Early drainage is recommended in addition to the use of the drug.—*Streptomycin in the treatment of tuberculous sinuses, R. H. Davis, Lancet, November 26, 1949, 2: 982.—(A. G. Cohen)*

Streptomycin for Pulmonary Tuberculosis.—Streptomycin was used in the treatment of 352 cases of nonmiliary pulmonary tuberculosis. A favorable effect on constitutional symptoms was practically constant and often spectacular but was unrelated to improvement in the pulmonary condition. The amount of expectoration decreased in many cases within one or two weeks; cough was less frequently influenced. Roentgenographically, fresh nodular infiltrations responded best. Of 136 patients in whom such lesions predominated, there were 28 with "total cure," 56 with complete clearing, 38 with considerable clearing, and 14 with a mediocre or unfavorable result. Fresh, nodular, pericavitary infiltrations and bronchogenic spreads often responded rapidly to treatment. Generalized nodulations without cavitation sometimes showed a delayed response as late as three to four months after the start of treatment. Some failures were encountered among these cases, especially those of the macronodular type. Healing was almost always incomplete in cavitary disease. Of 122 cavities, 3 closed completely as confirmed by tomography, 99 showed considerable diminution in size, 20 remained unchanged. The effect of streptomycin was irregular, unpredictable, and disappointing in fibrocavernous disease. Of 36 patients in this category 2 had good results, 11 showed moderate improvement, and 23 were failures. The response was unimpressive in patients with homogeneous lobar consolidation and undetectable in those with consolidated, retracted lobes and bronchial stenosis. Streptomycin was useful for complications following intrapleural pneumonolysis and for bronchopleural fistula. Three cases of early infiltrates had rapid and persistent clearing after three weeks of treatment. Equally good results were seen in serious forms of primary tuberculosis. Daily doses of streptomycin, 1.0 Gm., were found as effective as higher doses.

Loss of drug effectiveness after two to three months could not always be attributed to the emergence of resistant tubercle bacilli. The incidence of relapse in cases with good result was low; it occurred in 12 cases of which 8 responded to re-treatment.—*La streptomycine dans le traitement de la tuberculose pulmonaire non miliare, A. Duroux, Verges, Marty, Ablard, & Moreau, Rev. de tuberc., 1949, 13: 617.*—(*V. Leites*)

Aerosol PAS for Tuberculosis.—Ten cases which fulfilled the following criteria were selected for treatment: (*1*) bilateral tuberculosis, (*2*) little likelihood of becoming suitable for collapse therapy by means of bed rest alone, and (*3*) moderately advanced disease showing no further improvement with conservative therapy. In 9 cases there were tension cavities, which are considered evidence of bronchial involvement. Bronchoscopic examination in 3 cases showed lesions. The patients received daily 10 Gm. of para-aminosalicylic acid (PAS) orally; this was given in 5 divided doses at four-hour intervals. The drug was omitted one day each week. In addition, 2 aerosol treatments with 1 cc. of 20 per cent PAS were given daily. In 4 cases the cavities closed and in 2 there was improvement. In 2 there was failure; in the remaining 2 the period of observation was insufficient. In other cases, ulcerative lesions of the mouth and larynx were improved.—*Para-aminosalicylic acid as an aerosol in the treatment of respiratory tuberculosis, A. E. K. Salvi, Tubercle, October, 1949, 30: 223.*—(*A. G. Cohen*)

Streptomycin Sensitivity of Tubercle Bacilli—Tubercle bacilli grown in Tween 80-albumin liquid medium were tested for sensitivity to streptomycin. All strains were derived from tuberculous patients before, during, and after treatment with streptomycin. Strains isolated before treatment showed no real difference in sensitivity. Resistant strains fell into 3 groups: (*1*) those 4 times less sensitive than the standard strain, (*2*) those 128 times less sensitive, and (*3*) those which grew on at least 1,000 units of streptomycin per cc. There was usually an abrupt change from nonresistance to one of the 3 categories of resistance. Once in one of these groups, the organisms' resistance usually did not change with further treatment. Pretreatment resistance may mean that the patient was infected with bacilli from a treated individual. Increase in resistance often may not be demonstrated unless 2 or more strains are tested.—*Tests for streptomycin sensitivity of tubercle bacilli in Tween 80 albumin liquid medium, D. A. Mitchison, Lancet, October 15, 1949, 2: 694.*—(*A. G. Cohen*)

Chloromycetin for Pneumonia in Children.—Thirty-three cases of pneumonia in infants and children were treated with chloromycetin; 18 of these were pneumococcal and the others were caused by various organisms. Twenty were acutely ill; 8 needed oxygen. Chloromycetin was given orally in daily doses of 50 to 220 mg. per Kg. This was given in divided doses every three hours and treatment extended over two to six days with an average of 4.8 days. The total dose was 3.4 to 11.3 Gm. with an average of 5.9 Gm. Usually, the contents of the capsule were dissolved in a vehicle in order to disguise the taste; there were no toxic symptoms. The results were good in 25, fair in 7, and poor in one. In 20 cases, the temperature fell to normal in eighteen hours. The pathogens rapidly disappeared from the sputum and the roentgenographic signs improved. The results were comparable to those achieved with aureomycin.—*Chloromycetin in the treatment of pneumonia in infants and children, A. Recinos, Jr., S. Ross, B. Olshaker, & E. Trimble, New England J. Med., November 10, 1949, 241: 733.*—(*A. G. Cohen*)

Intratracheal Penicillin Atomization.—Pulmonary suppuration was treated by atomizer instillation of 100,000 to 500,000 units of crystalline penicillin daily through the partially anesthetized larynx. Following pontocaine anesthetization, the penicillin in 5 cc. of isotonic saline solution was sprayed between the vocal cords during inspiration and expiration. The position of the patient was determined by the location of the sup-

purative process, thus obtaining a better volume and concentration of the drug than can be obtained by the usual aerosol procedures.—*Intratracheal atomization in the treatment of infectious diseases of the respiratory system (a preliminary report), P. L. Farinas, O. S. de Bustamante, L. M. Lott, & R. Revuelta, Dis. of Chest, May 1948, 15: 546.*—(*E. A. Rouff*)

LABORATORY STUDIES

Phagocytic Activity of Bone Marrow.—A comparison between cultures of human bone marrow shows that the marrow of tuberculin-sensitive individuals has a far greater phagocytic activity than the marrow of individuals not sensitized to tuberculin. Marrow cultures inoculated with tubercle bacilli have a high phagocytic activity in the first 48 to 60 hours but this capacity is less when more virulent strains of bacilli are used. Thus tubercle bacilli of the avian type are phagocyted in greater amounts than bacilli of bovine or human strains. The presence in the cultures of serum originating from infected or noninfected individuals does not influence the phagocytic properties of the culture, but the marrow of infected individuals retains *in vitro* a specific hypersensitivity to tuberculin. Theoretically it is possible that the greater phagocytic activity and the hypersensitivity to tuberculin of the marrow of tuberculous individuals are both an expression of the defensive character of allergy.—*Il comportamento verso il bacillo tubercolare del midollo osseo umano coltivato in vitro, G. T. Sicca, Lotta contro tuberc., February, 1949, 19: 210.*—(*A. Mochi*)

Plasma Factor in Cytolysis by Tuberculin.—There is present in the plasma of tuberculous humans a heat-labile, non-dialyzable component which is responsible for the *in vitro* lysis of washed leukocytes by tuberculoprotein. Further properties of this lytic-producing substance are its stability at 10°C. for at least seven days and its precipitation with the globulin fraction of the plasma proteins.—*Nature of the plasma factor responsible for in vitro lysis of leukocytes by tuberculoprotein, J. M. Miller, C. B. Favour, B. A. Wilson, & M. A. Umbarger, Proc. Soc. Exper. Biol. & Med., June, 1949, 71: 287.*—(*F. B. Seibert*)

Staining of Tubercle Bacilli.—A new staining method for tubercle bacilli in pathological materials has been shown to be specific in smears of 200 varied specimens. The slides are fixed with Cornoy's fluid, drained, then half covered with a saturated solution of sudan black B in 70 per cent alcohol. The stain is lighted and allowed to burn out before swilling the slide with water. Differentiation is accomplished by immersion in acetone for two minutes. The smear then is rinsed with water and counterstained with a 1 per cent aqueous solution of pyronin for 10 to 60 seconds. With this method, human tubercle bacilli from human lesions are strongly stained black. Sometimes they stain evenly; commonly they have a rather barred appearance, even when Ziehl-Neelsen staining shows no beading. Human, bovine, and avian tubercle bacilli in culture are not stained. For clinical use, the chief advantages of this method as compared with Ziehl-Neelsen are: (*1*) that the time taken from receipt of the sputum to the microscopic examination is only four minutes and (*2*) that other acid-fast bacilli are not demonstrated. The tubercle bacilli are easy to recognize microscopically; they are black instead of red, but have a rather characteristic morphology because of the beading.—*The staining of tubercle bacilli with sudan black B, H. L. Sheehan & F. Whitwell, J. Path. & Bact., April, 1949, 61: 269.*—(*H. J. Henderson*)

Staining of Much's Granules.—A simple combination of Fontes' and Cerqueira's methods gives quite satisfactory results in the staining of Much's bodies. The materials used include Ziehl's solution, 2 per cent ethyl chloride, iodine solution, 5 per cent sodium thiosulfate solution, and 1 per cent brilliant green aqueous solution. The granules assume a dark, blackish color, which makes them stand out against the red bodies of the bacilli in the green background.—*Técnica de coloração das granulações de Much do Mycobacterium tuberculosis,*

P. de Góis, Rev. brasil. de tuberc. March–April, 1949, 17: 187.—(A. A. Moll)

Incoagulability of Intrapleural Blood.—Intrapleural blood, whatever the cause of the hemorrhage, in most cases does not coagulate and resorbs in eight to ten days' time. Samples of blood taken after thoracentesis and kept for more than two months in test tubes do not show any tendency to coagulate. Even if fresh blood is added no coagulation takes place until dilutions of one to 10 or more are reached. Pleural fluid alone does not show this property. Intrapleural hemorrhages or instillations of blood as a rule do not cause fever, particularly if the pleura is already altered by inflammatory processes. In 2 cases hemorrhage into the pleural cavity was followed by resorption of a previously therapy-resistant empyema; instillation of blood into the empyema cavity was followed by success only in one case of 3. In no case, however, did the blood coagulate. The theory is advanced that blood in contact with the pleura undergoes a change due to some unknown "incoagulability factor".—*Contributo allo studio della incoagulabilità del sangue nel cavo pleurico, F. Vergani, Gior. ital. tuberc., November–December, 1948, 2: 391.—(A. Mochi)*

Pneumothorax and Blood Elements.—Artificial, closed pneumothorax was maintained in a total of 13 unanesthetized dogs for periods of 11 to 75 days by repeated insufflations of room air. Respiratory compensation was adequate and anoxic anoxia was not present. In a few days the hematocrit, oxygen capacity, and red blood cell count values rose above those of the control period and reached a maximum in two to four weeks. At the end of the pneumothorax period, these values returned to the control level in about a week. No evidence of erythropoetic stimulation was obtained since the reticulocyte percentage and hemoglobin production remained within the control ranges. In addition, the presence of the spleen is not essential to the hematocrit increase. Plasma volumes decreased as the hematocrit values increased and this inverse relationship was maintained throughout the course of the procedure. Plasma protein percentage showed no consistent change. It is concluded that the increased values of the formed elements of the blood during pneumothorax is a result of hemoconcentration caused by loss of fluid from the vascular bed. (Author's summary).—*Changes produced in hematocrit value, hemoglobin and plasma volume by repeated artificial pneumothorax, W. C. Grant, Am. J. Physiol., November 1, 1949, 159: 394.—(G. C. Leiner)*

PUBLIC HEALTH AND EPIDEMIOLOGY

Tuberculosis after Negative Microfilm.—Active pulmonary tuberculosis was diagnosed in 23 patients who previously had had negative reports of 70 mm. roentgenograms of the chest. Respiratory symptoms presumably caused by pulmonary tuberculosis were present in 9. These patients were "bacteriologically diagnosed" before December 1, 1948. The survey films were made between May 5 and August 25, 1948. Such occurrences may arise because the disease develops acutely. In addition, portions of the lung fields are obscured by the heart, diaphragm, and skeleton in conventional roentgenograms, and extrapulmonary tuberculosis usually is not revealed by such films.—*Active pulmonary tuberculosis following negative 70 mm. film impressions in Minneapolis, mass chest X-ray survey, W. Roemmich, J. Lancet, 69: 122.—(H. J. Henderson)*

Small-film Screening in Major Cities.—City-wide tuberculosis surveys can be conducted with relative economy of money and effort. The concentration of personnel, machinery, and educational devices provides quicker and more reliable results than studies in sparsely settled areas. In the United States there are 92 cities with populations of over 100,000 containing together more than 30,000,000 inhabitants of fifteen years or over. If roentgenograms of this group can be made within a short period, 30.7 per cent of the population that must be covered will have been examined. The Tuberculosis Control Division of the Public Health Service proposes a plan for such screening in these cities, using 12 to 14

microfilm units in any one city at a given time. The plan anticipates screening some 30,000,000 people in a reasonably short time, preferably five years.—*Role of the roentgenologist in a proposed plan for small-film screening of the inhabitants of major cities, T. F. Hilbish, Radiology, February, 1949, 52: 248.*—(*W. J. Steininger*)

Tomography in Tuberculosis.—Because of the shortage of institutional beds for tuberculosis, it has become necessary to carry out more extensive studies in outpatient departments. Tomography has been of great value in this respect. One hundred cases were analyzed from the viewpoint of determining what additional data were supplied by tomography. There were 12 groups: (*1*) questionable cavity in conventional film with cavity in tomogram, 24 cases; (*2*) conventional film not suspicious of cavity, shadowing fairly heavy with or without positive sputum but tomogram showed cavity, 9 cases; (*3*) questionable cavity in conventional film, no cavity in tomogram, 32 cases; (*4*) collapse in conventional film but cavity seen within collapsed lung in tomogram, 4 cases; (*5*) collapse in conventional film with dilated bronchus in tomogram, 4 cases; (*6*) surgical collapse in conventional film and blocked cavity in tomogram, 1 case; (*7*) conventional film showed collapse therapy and no cavity but sputum was positive; tomogram revealed cavity, 3 cases; (*8*) conventional film as in (*7*); tomogram showed no cavity although bronchoscopy showed lesion, 6 cases; (*9*) small area of disease in conventional film and larger area in tomogram, 5 cases; (*10*) conventional film negative, sputum positive; tomogram revealed a tuberculous focus, 1 case; (*11*) conventional film showed pleural effusion; tomogram disclosed disease in underlying lung, 3 cases; (*12*) in miscellaneous cases, tuberculoma, et cetera; tomogram yielded additional information, 8 cases. Groups (*1*), (*2*), and (*3*) comprised the bulk of the cases. It is seen that preliminary tomography did not avoid hospital admission in groups (*1*) and (*2*) but did avoid it in 25 cases of 32 in group (*3*).—*The application of tomography to the problem of sanatorium bed space, N. Wynn-Williams, Tubercle, June, 1949, 30: 122.*—(*A. G. Cohen*)

Tuberculosis in Medical Students.—The incidence of tuberculosis in medical students is high and may be related to some of the students' activities such as autopsy work. This usually is considered a hazard from the point of view of tuberculosis.—*Medical students and tuberculosis, C. H. Schmidt, J. Lancet, April, 1949, 69: 128.*—(*H. J. Henderson*)

Results of BCG Vaccination in Brazil.—Of 4,649 infants vaccinated with BCG at Rio de Janeiro, 2,000, all successfully vaccinated, were followed. During the first year of life, 33.25 per cent of the group were known to be exposed to tuberculosis at home; exposure was suspected in 26.16 per cent. During the second year, 30.87 and 42.76 per cent, respectively, had known and suspected exposure. No accurate information was available for the remainder. During the first year, 9 cases (0.51 per cent) of tuberculosis developed and during the second year, 15 cases (1.93 per cent) appeared. In both years the incidence of tuberculosis was higher among children with known exposure to the disease: 1.53 per cent in the first year and 3.62 per cent in the second year. The rise in the second year supports the view that a certain proportion of vaccinated children gradually lose immunity. Deaths from tuberculosis totaled 5: one in the first year, 3 in the second, and one in the third year. In 4 of these cases there was known exposure and in one, exposure was suspected. The proportion of tuberculin-anergic cases rose from 35.05 per cent in the first year to 49.35 per cent in the second year, and of hyperergic cases from 8.44 to 10.92 per cent. The proportion of mesergic cases decreased from 56.5 to 39.71 per cent.—*Resultados de investigação realizada em 2,000 crianças vacinadas pelo BCG, A. MacDowell Filho, Rev. brasil. de tuberc., March–April, 1949, 17: 189.*—(*A. A. Moll*)

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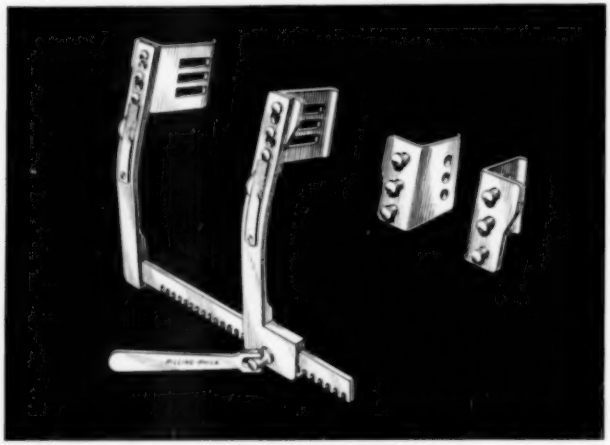

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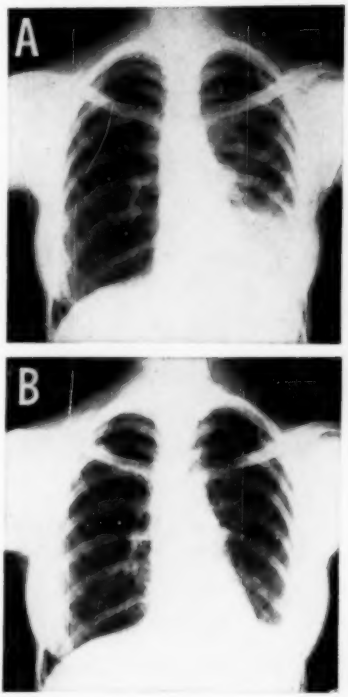

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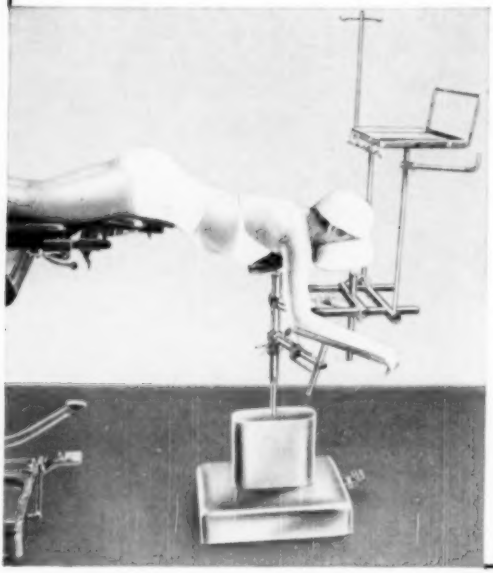

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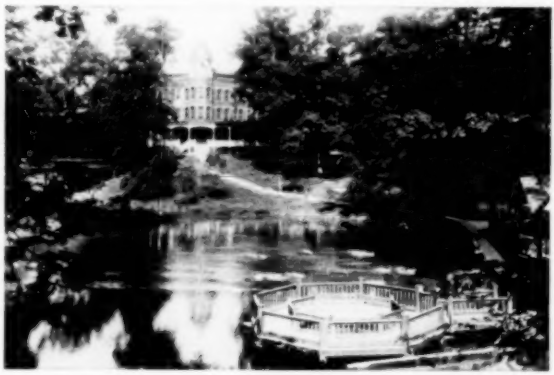

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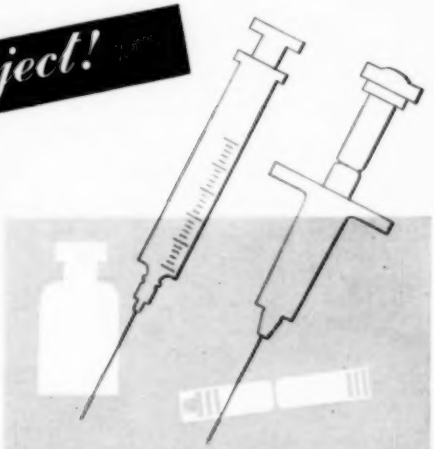

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Ref.: Hinshaw, H. C. and McDermott, W.: Report to the Clinical Subcommittee of the Committee on Medical Research and Therapy, Amer. Review of Tuberculosis, 1950, 61, 157.

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